

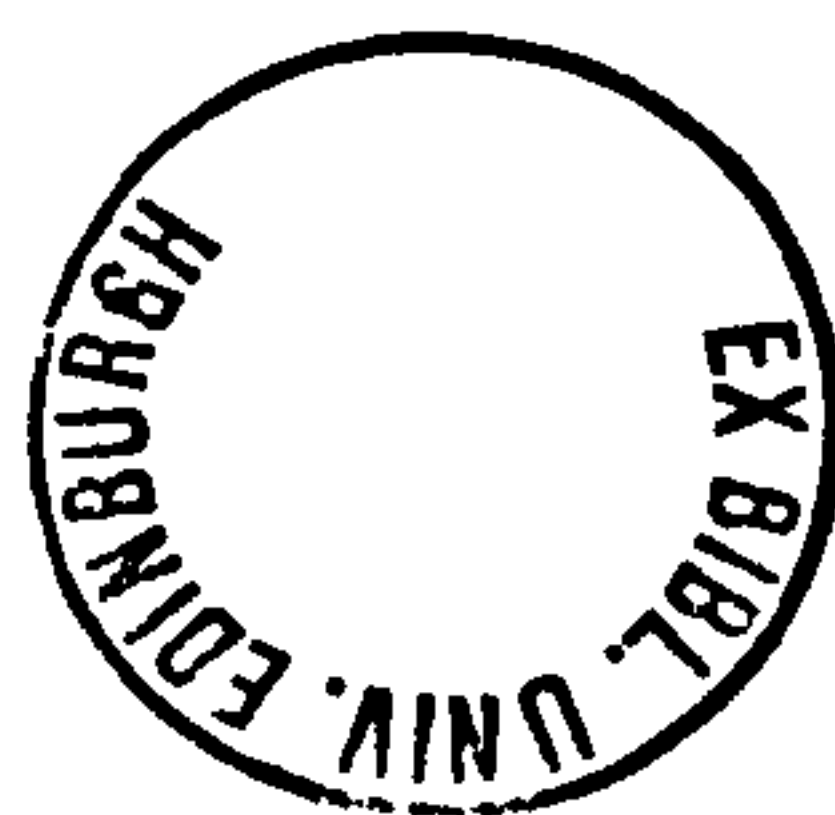
CLINICAL AND GENETIC STUDIES OF
VON RECKLINGHAUSEN NEUROFIBROMATOSIS

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To my parents in gratitude

for their support and encouragement

The main body of data presented in this thesis was collected whilst the author was working as a Clinical Research Officer in the Department of Medical Genetics and Section of Neurology, University of Wales College of Medicine, Cardiff, between October 1983 and February 1986. Since that time the author has worked at Northwick Park Hospital, first in the Division of Inherited Metabolic Diseases, Clinical Research Centre (until July 1987) and then as Senior Registrar in Medical Genetics at the Kennedy Galton Centre. Throughout this time the author has acted as clinical co-ordinator for laboratory-based studies on neurofibromatosis which have continued in the Medical Genetics Department in Cardiff.

This thesis is submitted for the Degree of Doctor of Medicine at the University of Edinburgh. It has not previously been submitted at this or any other university. Unless stated, the work reported was performed by the author without assistance and the thesis was compiled by the author alone.

..... Candidate

..8th May..1989.. Date of submission

CONTENTS

	Page
Contents	1
List of Tables	5
List of Figures	7
Acknowledgements	9
Abstract of thesis	11

PART 1

CLINICAL AND GENETIC STUDIES OF VON RECKLINGHAUSEN NEUROFIBROMATOSIS IN SOUTH EAST WALES

<u>Chapter 1</u> Von Recklinghausen neurofibromatosis (NF-1) - Literature review and aims of present study	13
1. Introduction	13
2. Literature review:	14
A. Clinical aspects	
B. Genetic aspects	
C. Pathological findings in NF-1	
D. The different forms of neurofibromatosis	
E. Differential diagnosis	
F. Pathogenesis	
3. Aims of the present study	38
<u>Chapter 2</u> The South East Wales population study - ascertainment and assessment of cases	39
1. The study area	39
2. Patient ascertainment	39
3. Patient assessment	41
4. Analysis of results	44
<u>Chapter 3</u> Prevalence of NF-1 and genetic analysis of the South East Wales population	45
1. Outcome of clinical assessment of ascertained cases	45
2. Outcome of analysis	49
A. Pedigree analysis	
B. Disease prevalence	
C. Estimation of fitness in NF-1	
D. Mutation rate of the NF-1 gene	
E. Analysis of parental age and mutation	
F. Parental effect on severity	
3. Discussion	59

<u>Chapter 4</u>	The major and minor features of NF-1	65
1.	Introduction	65
2.	The major defining features of NF-1	66
	A. CAL spots and freckling	
	B. Peripheral neurofibromas	
	C. Lisch nodules	
	D. The age of appearance of the major defining features	
	E. Discussion	
3.	Minor features of NF-1	83
	A. Macrocephaly	
	B. Short stature	
<u>Chapter 5</u>	The complications of NF-1.	85
1.	Introduction	85
2.	The complications of NF-1	86
	A. Plexiform neurofibromas	
	B. Intellectual handicap	
	C. Neurological complications	
	D. Skeletal complications	
	E. Malignancy with established disease association	
	F. Endocrine complications	
	G. Cardiovascular complications	
	H. Symptomatic neurofibromas	
	I. Congenital glaucoma	
	J. Overview of frequency of complications	
3.	Other diseases observed in the study population.	111
4.	Disease mortality.	113
<u>Chapter 6</u>	Patient care and genetic counselling in NF-1	118
1.	Introduction	118
2.	Patient care in the South East Wales population	119
	A. The diagnosis of NF-1	
	B. Patient care and genetic counselling	
3.	Guidelines for the management and counselling of NF-1 sufferers	124
	A. Genetic counselling	
	B. Patient management	
PART 2		
GENETIC LINKAGE STUDIES OF VON RECKLINGHAUSEN NEUROFIBROMATOSIS		
<u>Chapter 7</u>	Introduction to human gene mapping and review of literature pertinent to the possible localisation of the NF-1 gene.	131
1.	Introduction	131

2. Recombinant DNA technology and the study of human genetic disease	131
A. Restriction endonucleases	
B. Cloning of DNA fragments	
C. Gene libraries	
D. Application of DNA technology to localisation and isolation of genes.	
3. Methods of human gene mapping	138
A. Family linkage studies	
B. Somatic cell hybrids	
C. Cytogenetics and gene mapping	
4. Review of previous reports pertaining to the localisation of NF-1 gene.	146
<u>Chapter 8</u> Genetic linkage studies of NF-1 and chromosome 19 markers linked to myotonic dystrophy (DM).	149
1. Introduction	149
2. Methods	150
A. Family ascertainment	
B. Typing of C3 and APOC2 polymorphisms	
C. Secretor typing	
D. Lu and PEPD typing	
E. Linkage analysis	
3. Results	161
4. Conclusions	164
<u>Chapter 9</u> An exclusion map for NF-1	167
1. Introduction	167
2. Outcome of analysis of other genetic markers and NF-1	167
A. Protein polymorphisms	
B. Studies of other DNA polymorphisms	
C. Collaboration with other centres	
3. An exclusion map for NF-1	171
A. Background	
B. Description of the 'EXCLUDE' program	
C. Origin of linkage data	
D. The exclusion map for NF-1	
<u>Chapter 10</u> Sub-chromosomal localisation of the NF-1 gene	179
1. Introduction	179
2. Linkage analysis of chromosome 17 markers	180
A. Studies in the Cardiff family panel	
B. The combined NF-1 linkage consortium data set	
3. Physical mapping studies of the NF-1 region on chromosome 17	185
A. Cytogenetic studies in NF-1 patients	

B. Physical mapping studies using hybrid cell lines and pulse field gel electrophoresis	
C. Analysis of tumour DNA from NF-1 patients	
4. Conclusions	189
REFERENCES	191
APPENDICES	213
<u>Appendix A</u> Clinical summaries of families studied	213
Section 1. NF-1 families	213
Section 2. Index cases with only CAL spots	367
Section 3. Index cases with other forms of neurofibromatosis	369
Section 4. Index cases with other diagnoses	377
<u>Appendix B</u> Genotypes for C3, APOC2 and Se polymorphisms in the 9 families studied	386
<u>Appendix C</u> 1. Questionnaire used for interviewing patients	397
2. Computer data entry form.	408

TABLES

	Page
1-1 The clinical features of NF-1	15
1-2 Prevalence of major disease complications in previous large surveys of NF-1	17
1-3 Summary of previous estimates of prevalence and mutation rate for NF-1	22
1-4 Classification of the different types of neurofibromatosis proposed by Riccardi (1982)	28 + 29
3-1 Exclusions based on clinical features in 24/119 cases originally ascertained	47
3-2 Breakdown of pedigree information based on parental details for 48 cases with no family history of NF-1	51
3-3 Distribution of 33 new mutations by birth order	55
3-4 Mean parental ages of NF-1 cases classified as new mutations	57
3-5 Mean severity grade of 124 cases of NF-1 classified by disease status of parents	58
4-1 Age related frequency of CAL spots and freckling	70
4-2 Percentage of cases with each of the major defining features by age	71
5-1 Frequency of previously recognised complications of NF-1	87
5-2 Frequency of CNS tumours and other malignancy associated with NF-1	98
5-3 Other diseases observed in the study population	112
5-4 Causes of death in 25 deceased affected relatives	116 + 117
6-1 The 135 affected individuals divided according to who had made the diagnosis	120
6-2 The 41 affected individuals aged <18 years divided according to who had made the diagnosis	121
6-3 Reason for hospital follow-up in 30/135 individuals in study area	123
6-4 Frequency of complications for counselling purposes	126
8-1 Expected ABH antigens in saliva	159
8-2 Agglutination results in Secretor typing	160

8-3	Lod scores between NF-1 and APOC2, C3 and Se	163
9-1	Linkage analysis between NF-1 and marker loci discussed in Sections 9-2 A and B	169
9-2	Combined linkage data for NF-1 used for exclusion map shown in Figure 9-2A	175
10-1	Outcome of linkage analysis of 16 chromosome 17 DNA markers in the Cardiff NF-1 family panel	181
A-1	Study numbers of affected individuals in South East Wales cross-referenced with pedigree numbers, mode of inheritance and complications.	216
A-2	Study numbers cross-referenced with pedigree numbers, for other affected family members specifically mentioned in the main text.	219

FIGURES

	Page
2-1 Map of the study area in South East Wales	40
3-1 Outcome of follow-up of the 119 cases originally ascertained as having NF-1	46
4-1 CAL spots	68
4-2 Average number of CAL spots per individual in 153 cases of NF-1	69
4-3 Axillary freckling	72
4-4A,B,C The development of the cutaneous features of NF-1 with age	74
4-5 The comparison of number of cutaneous neurofibromas with age	75
4-6 Lisch nodules	76
4-7 Variation in presence/absence of Lisch nodules with age	79
4-8 Presence/absence of major defining features in the offspring of affected individuals by age	81
5-1A,B,C Plexiform neurofibromas	90
5-2A,B Scoliosis	101
5-3 Lambdoidal suture defect	103
5-4 Vertebral scalloping	105
5-5 Difference in the estimated prevalence of NF-1 with age	115
6-1 Age range of presentation and frequency of major NF-1 complications	128
6-2 Proposed plan for organisation of specialist neurofibromatosis clinics	130
7-1 Detection of a RFLP by a DNA probe	137
7-2 Apparent co-segregation of NF-1 and DM (Ichikawa <i>et al</i> 1981)	148
8-1 Diagrammatic representation of the procedures involved in RFLP analysis	151
8-2 Southern blotting	155
8-3 A,B APOC2 and C3 RFLPs studied	162
8-4 Physical and genetic map of chromosome 19	166

9-1A,B,C	Examples of the likelihood distributions produced by the program 'EXCLUDE'	173
9-2 A,B	Exclusion maps for NF-1	176
10-1	Genotypings of chromosome 17 markers in family NF17, a critical recombinant family in establishing flanking markers	183
10-2	Physical and genetic map of chromosome 17	186
A-1:		385
A -	Case NNF14, who had steatocystoma multiplex	
B -	Case NNF15, who had LEOPARD syndrome	
C -	Atypical areas of CAL pigmentation in case NNF16 in whom no diagnosis was reached.	

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ABSTRACT

A population-based study in South East Wales (population 668,100) identified 69 families with 135 affected members with von Recklinghausen neurofibromatosis (NF-1), giving a disease prevalence of $20/10^5$ of population. In these families penetrance of the NF-1 gene was 100% by the age of five years. 41/135 cases were judged to represent new disease mutations and the mutation rate was estimated to lie between 3.1×10^{-5} and 10.4×10^{-5} . A parental age effect for new mutations was not demonstrated, nor was a maternal effect on disease severity.

The clinical features and natural history of NF-1 in this cohort were used to derive data for genetic counselling and recommendations for the management of affected individuals. For counselling purposes the complications of NF-1 can be usefully divided into 4 categories (the frequency of each, based on this study, are shown in parentheses): intellectual handicap (33% overall, moderate/severe retardation 3.2%, minimal retardation/learning difficulties 29.8%); complications developing in childhood and causing lifelong morbidity, e.g. facial plexiform neurofibromas, scoliosis, pseudoarthrosis (8.5%); 'treatable' complications which can develop at any age, e.g. benign disorders of the nervous system, visceral and endocrine tumours, renal artery stenosis (15.7%) and malignant or CNS tumours (4.4-5.2%).

The study population indicates that sufferers are not being diagnosed sufficiently early, nor receiving appropriate follow-up and counselling. It is recommended that patients with NF-1 have regular clinical assessments to monitor for the development of complications, although none occur often enough to warrant biochemical or radiological screening. As many of the complications develop early in life, children should have biannual review; in adults, unless a particular complication indicates more frequent review,

annual clinical examination is sufficient.

Alongside the population survey, genetic linkage studies were undertaken in selected large families to determine the chromosomal localisation of the NF-1 gene. At the outset of this work, two families had been reported in which NF-1 and Myotonic Dystrophy (DM) appeared to co-segregate, suggesting that the two genes were closely linked and on chromosome 19. However, linkage studies of 3 chromosome 19 markers linked to DM showed significantly negative lod scores, therefore excluding this possibility. Other chromosomes were then studied using random unique sequence DNA probes and samples from the largest families were made available to collaborators in the USA for linkage studies using possible candidate genes (β nerve growth factor and oncogenes). No marker studied showed evidence of linkage.

The negative data were used to produce an exclusion map for NF-1, using the computer program 'EXCLUDE'. The presentation of this work was one of the factors which precipitated the formation of an international consortium for NF-1 linkage in February 1987; the first task of the consortium was to produce an expanded exclusion map. A small positive lod score for a marker on chromosome 17, taken with the exclusion data, showed that NF-1 was seven times more likely to be on chromosome 17 than any other chromosome; this was rapidly confirmed by two North American groups, one of which was using samples from the 5 largest families presented in the thesis. Subsequent linkage analysis of pericentromeric chromosome 17 markers in the Welsh family panel showed no evidence of non-allelic heterogeneity and identified closely linked flanking markers for the NF-1 gene suitable for prenatal/presymptomatic diagnosis. The chromosomal localisation of NF-1 represents a major step towards the eventual understanding of the disease pathogenesis and the development of possible treatments.

PART 1

CLINICAL AND GENETIC STUDIES OF VON RECKLINGHAUSEN

NEUROFIBROMATOSIS IN SOUTH EAST WALES

CHAPTER 1 Von Recklinghausen neurofibromatosis - literature review
and aims of present study

1. Introduction

"So you see that it is not an idle whim of mine to talk and write so often about these phakomatoses*, but that the study of them can be of great advantage for our patients and of great value for our knowledge."

Van der Hoeve,
Doyne Memorial Lecture, 1932.

Von Recklinghausen or type 1 neurofibromatosis (NF-1) is one of the commonest autosomal dominant disorders of man; Crowe *et al* (1956) estimated that the birth incidence may be in the region of 1/2500-3300. The major features of the disease are cafe au lait (CAL) spots, peripheral neurofibromas and Lisch nodules (pigmented iris hamartomas). The morbidity and mortality caused by NF-1 is largely dictated by the occurrence of its complications; these are numerous and can involve any of the body systems.

In embarking on the present study of NF-1, the primary interest was in mapping the disease gene by family linkage studies; this work is presented in part 2 of this thesis. Reviewing the literature at the outset, it became apparent that there were many unanswered questions about various clinical and genetic aspects of the disease; there had been no major survey of NF-1 in the British population. Alongside linkage studies, a population-based study of the disease in South East Wales was therefore undertaken.

* Van der Hoeve coined the term phakomatosis in 1923 to epitomise NF-1 and tuberous sclerosis; "phakos" is the Greek for birth mark.

In this chapter an overview of the NF-1 literature is presented and the unresolved questions addressed by the population study outlined.

2. Literature review

(A) Clinical aspects - The clinical features of NF-1 are summarised in Table 1-1. The disease complications are based on the findings of previous large population- or hospital-based surveys (Borberg 1951; Crowe *et al* 1956; Brasfield & Das Gupta 1972; Riccardi & Kleiner 1977; Bader & Miller 1978; McKeen *et al* 1978; Carey *et al* 1979; Samuelsson 1981; Riccardi & Eichner 1986).

From a historical viewpoint, the first descriptions are of adult patients with multiple dermal tumours found in the eighteenth century literature (Akenside 1768; Ludwig & Tilesius 1793). By 1849 Smith was able to cite 75 references in a review of the literature; he presented two further cases and postulated, although was unable to prove, that the tumours arise from the fibrous connective tissue sheath of small nerves. Thirty-three years later, von Recklinghausen (1882) demonstrated that this was indeed the case and named the tumours neurofibromas. Von Recklinghausen also emphasised that one of his two cases had an affected sibling and realised the disease was not acquired. Following this important work, the disease became known as von Recklinghausen's disease.

Von Recklinghausen (1882) also described "innumerable brown pigmentation spots" in one of his cases, but did not appreciate their significance. The credit for this falls to Marie & Bernard (1896)

Table 1-1 The clinical features of NF-1

<u>Major defining features</u>	b) <u>Skeletal</u>
CAL spots ± axillary freckling	Scoliosis
Peripheral neurofibromas	Lateral thoracic meningocele
Lisch nodules	Lambdoidal suture defects
	Pseudoarthrosis of distal long bones
<u>Minor features</u>	c) <u>Genitourinary</u>
Macrocephaly	Neurofibromas
Short stature	Pelvic rhabdomyosarcoma
	Wilms tumour*
<u>Complications (by body system)</u>	d) <u>Endocrine</u>
(a) <u>Nervous system</u>	Phaeochromocytoma
Plexiform NFS*	Duodenal carcinoid
Intellectual handicap	Premature/delayed puberty
Spinal NFS	e) <u>Cardiovascular</u>
Gliomas (particularly optic nerve)	Renal artery stenosis
Epilepsy	Cerebrovascular disease
Peripheral nerve malignancy	f) <u>Respiratory</u>
Aqueduct stenosis	Neurofibromas of oral cavity, larynx, mediastinum
Co-ordination problems	g) <u>Gastrointestinal</u>
Acoustic neuroma*	Visceral neurofibromas
Meningioma*	h) <u>Haematopoietic</u>
Hypsarrythmia*	Atypical forms of childhood leukaemia
Neuroblastoma*	

* Indicates some doubt whether definite increased frequency

** NFS = neurofibromas

and Chauffard (1896). Although it was not until the study of Crowe *et al* (1956) that it was accepted that CAL spots were the first manifestations of the disease to appear, some authors prior to this had described young patients with only CAL spots as having a "forme fruste" of the disease (Parkes Weber 1909). Much later, Crowe (1964) reported that axillary freckling was a clinical feature unique to NF-1.

Abnormalities of the iris in NF-1 were first reported clinically by Waardenburg in 1918; he described small wart-like iris lesions which he presumed to be neurofibromas. Goldstein & Wexler (1930) demonstrated multiple melanocytic tumours of the iris in the eyes of a patient with NF-2 at necropsy. In 1937 Lisch reported 3 cases of NF-1 all of which had nodular lesions of the iris which had a distinct appearance. He concluded that they were pathognomic of the disease and that if the irides of more NF-1 patients were examined, they would be a frequent finding. His hypothesis was not tested until 1981 and was then found to be correct; Lewis & Riccardi found Lisch nodules to be present in 56/61 (92%) of patients >5 years of age.

After the recognition of NF-1 as a distinct entity, clinical reports of complications affecting most of the body systems began to appear in the literature, but it was only with the first large clinical studies of Borberg (1951) and Crowe *et al* (1956) that the spectrum and prevalence of complications became more clear. Since that time there have been four further large surveys of patients with NF-1 (Brasfield & Das Gupta 1972; Carey *et al* 1979; Samuelsson 1981; Riccardi & Eichner 1986). The prevalence of the major complications found in the six studies are given in Table 1-2. There are limitations in all the studies. In some no clear distinction was

Table 1-2 Prevalence of major disease complications in previous large surveys of NP-1

Method of case ascertainment	Borberg (1951)	Crowe et al (1956)	Brasfield & das Gupta (1972)	Carey et al (1979)	Samuelsson (1981)	Riccardi & Elchner (1986)
	Hospital records	Hospital records & family studies	Hospital records	Genetic clinic records	Population based	Cases referred to special interest clinic
Diagnostic criteria adequate for excluding other forms of neurofibromatosis	Probably	No	Criteria not stated	No	No	Yes
No. of cases	84	223	110	131	74	238
Age range	All ages	All ages	All ages	All ages	Adults only	All ages
<u>Prevalence of complication (%)</u>						
Plexiform neurofibroma	16	16	NS†	NS	22	53
Intellectual handicap: Special school education	NS	NS	NS	NS	3	NS
IQ<70	10	NS	NS	10	NS	9
Epilepsy	7	NS	NS	6	3	3
CNS tumours	10	3	8	7*	0	15**
Acoustic neuroma	1	5	0	NS*	0	0
Spinal neurofibromas	5	0	4	1	0	5
Peripheral nerve malignancy	1	2	29	0	3	4
Scoliosis (all forms unless stated)	37	7	NS	5	3 (severe only)	29
Pseudoarthrosis	0	1	NS	0	3	3

† NS - prevalence of given complication not specified.

* Probably includes one case with BANF; ** All optic gliomas; 10% asymptomatic but diagnosed by CT.

made between NF-1 and the other forms of neurofibromatosis (Crowe *et al* 1956; Carey *et al* 1979; Samuelsson 1981). In others, the study sample was not population-based and therefore the frequency of complications may be an over-estimate, resulting from referral of more severely affected cases for specialist referral (Borberg 1951; Brasfield & Das Gupta 1972; Carey *et al* 1979; Riccardi & Eichner 1986). These differences in diagnostic classification and patient ascertainment mean that combining the findings of the various studies to give an overall estimate of complications is not possible and highlight the need for further studies looking at the frequency of complications in a series of patients with well-defined NF-1 ascertained in a relatively unbiased manner.

There is no specific preventive treatment available for any of the manifestations of NF-1; the majority of complications are treated in the same way as when they occur in isolation. In reviewing the NF-1 literature, it is apparent that all major clinical specialties are involved in the care of sufferers; reports of disease features in individual cases or groups of patients can be found in the journals of many different disciplines. The fact that most of the complications of NF-1 are individually rare has meant that no one specialty has had to take responsibility for the overall care of NF-1 sufferers. This undoubtedly has resulted in a lack of systematic clinical and laboratory investigation of the disease until relatively recently.

In the last decade, however, there has been an increasing awareness of the problems encountered by NF-1 sufferers and there has been pressure on the medical profession from the lay organisations for disease sufferers (e.g. the National Neurofibromatosis Foundation in the USA, formed in 1978; LINK in the UK, formed in 1981) to begin

to take an overview of the care of NF-1 sufferers, with particular regard to co-ordination of their medical care and the need for improved counselling about the clinical and genetic aspects of the disease. In the USA several centres have responded to this need by establishing neurofibromatosis clinics and have begun to formulate protocols for the assessment and follow-up of NF-1 sufferers. Riccardi, who established one of the first neurofibromatosis clinics, suggested the following protocol in a review article in 1981 (Riccardi 1981a):

"...all persons with neurofibromatosis or at risk for it should routinely undergo an extensive evaluation aimed at confirming the diagnosis, identifying complications, and monitoring progression. The evaluation should include the following procedures: intelligence-quotient measurement and psychological testing; electroencephalography; audiography; slit-lamp ocular examination; radiological skeletal surveying with special attention to the skull, optic foramina, and spine (and, in selected cases, internal auditory canals); cranial CT scanning with and without dye contrast, to include the orbits and optic chiasm; and measurement of 24-hour urinary excretion levels of epinephrine and norepinephrine."

At the outset of the present study no centre in the United Kingdom ran a specialist neurofibromatosis clinic. Discussions with colleagues in various disciplines suggested that some accepted the need to offer NF-1 sufferers regular follow-up although it was felt that screening investigations, such as those suggested by Riccardi, were unnecessary.

(B) Genetic aspects - The first family in which NF-1 occurred in more than one generation was probably that reported by Virchow in 1847. Thomson undertook a systematic genetic survey in 1900, demonstrating that the disease was familial in 30 of 77 reported cases. Preiser & Davenport (1918) studied the frequency of NF-1 in children of

affected individuals in the literature; they found that 50/115 (44%) were affected, with no difference in the sex distribution and many instances of male to male transmission; it was concluded that inheritance was autosomal dominant.

Subsequent authors accepted that at least in some families inheritance was autosomal dominant, but were confused by the high reported incidence of sporadic cases and failure to appreciate the significance of multiple CAL spots in relatives of affected individuals. Siemens (1926) suggested that inheritance was more often irregular dominant with frequent 'skipped' generations. Siemens concluded that the multiple CAL spots seen in relatives of index cases were an unrelated phenomenon; this was later to be proved wrong. In the same year, Fischer reviewed 466 previously reported cases and found that 81% of males and 64% of females were unmarried, that the age of marriage was high and fertility low. He suggested that mutation plays an important part in maintaining disease frequency and accounts for sporadic cases. Peyron *et al* (1937) thought the pattern of inheritance more complex and proposed 3 forms: monomeric dominant, monomeric recessive and a dimeric form in which there was one dominant gene and an additional modifying factor.

The question of mode of inheritance was resolved by the studies of Borberg (1951) and Crowe *et al* (1956). Both concluded that inheritance was autosomal dominant with high penetrance and that sporadic cases represent new gene mutations. Crowe and colleagues were the first to estimate the frequency of the disease (1/2,500-1/3,300) and mutation rate (1×10^4 per gamete per generation - the highest known for a human disorder). The subsequent studies of Sergeyev (1975) and Samuelsson & Axelsson (1981) found the disease to be less prevalent and the mutation rate more in line with

other disorders. The three studies are summarised in Table 1.3. The study of Crowe *et al* (1956) is the only one in which the genetic fitness of NF-1 sufferers, which is synonymous with relative effective fertility, has been estimated. They found the fitness of NF-1 sufferers to be approximately half of that of the normal population (0.53); the reduction was more marked in males than females.

In 1955 Penrose proposed that if mutations of disease genes are due to copy error at cell division, then as there are many more cell divisions in the male than female germ line, one would expect to see a paternal age effect - the fathers of new mutations being older than expected. This has been shown to be the case in several dominant diseases (e.g. achondroplasia, Apert syndrome; see Risch *et al* 1987 for most recent review). For NF-1, three studies have shown conflicting results. Samuelsson (1981) showed neither a birth order nor a parental age effect (with a paternal age effect one would expect sufferers to be born later than unaffected siblings). Sergeyev (1978) showed a birth order effect and a weak paternal age effect, the paternal age increase over population controls being 1.99 years ($p=0.03$). However, Riccardi *et al* (1984) showed a highly significant paternal age increase (3.15 years, $p<0.001$).

Another area of conflicting results has been in the investigation of maternal effect on disease severity. Miller & Hall (1978), studying 62 cases aged <18 years, found an increased severity in children born to affected fathers or new mutations. Two subsequent studies (Carey *et al* 1979; Riccardi & Wald 1987) failed to demonstrate this effect.

For genetic counselling the two main pieces of information required, in addition to mode of inheritance, are an estimation of

Table 1-3 Summary of previons estimates of prevalence and mutation
rate for NF-1

Study (year)	Method of ascertainment	Prevalence	Mutation rate (method)
Crowe, Neel & Schull (1956)	Surveys of general hospital admissions and state mental institutions	1/2500-3300	1.4-2.6x10 ⁻⁴ (direct) 0.7-0.9x10 ⁻⁴ (indirect) f=0.53
Sergeyev (1975)	Population sample of 16 year old youths	1/7800	4.4-4.9x10 ⁻⁵ (direct)
Samuelsson & Axelsson (1981)	Population based	1/4600	4.3x10 ⁻⁵ (direct)

the likely disease severity in affected offspring and at what age the disease features appear. All previous large studies of NF-1 have found that complications occur at random, even within families, the only exception being in identical twins (reviewed in Section F(i)). However, only one previous study has stated actual figures for risk of complications to use in genetic counselling. Riccardi *et al* (1979), based on the results of a hospital-based survey, state that for an affected person in a family brought to medical attention there is a 25% chance of moderate or severe complications and following from this, a 12.5% chance of similar problems in their offspring. No study has looked specifically at the age of appearance of the major defining features to derive an age when relatives at-risk, not demonstrating these features, can be reassured that they do not carry the gene.

(C) Pathological findings in NF-1 - Only the pathology of the major features of NF-1 and plexiform neurofibromas, a complication relatively specific to the disorder, are discussed here. For most of the complications, the underlying pathology is the same as when the lesion occurs in isolation; if this is not the case, the features specifically associated with NF-1 are discussed in Chapter 5, where the complications are individually reviewed.

(i) CAL spots - CAL spots are not unique to NF-1 sufferers. Crowe and Schull (1953) reported that 684/6853 (10%) institutionalised patients, who had no other NF-1 features, had 1-3 CAL spots and Kopf *et al* (1985) observed this number of CAL spots in 13.8% of patients attending a private dermatology clinic. There are no clinical differences in the appearance of the CAL spots in NF-1 sufferers and

the general population; it is the number of CAL spots which distinguish NF-1 sufferers.

Histologically there are two features which to some extent distinguish the CAL spots of NF-1 but neither is distinctive enough to act as a true diagnostic test. Johnson and Charneca (1970) reported that the CAL spots in NF-1 sufferers show an increase in DOPA-positive melanocytes, as did an axillary "freckle" from one of their cases. The control normal skin from NF-1 sufferers showed a less marked increase. In normal individuals with only one or two CAL spots, these authors found no alteration in number in the normal skin but a decrease in melanocytes in the CAL spots. They argued that these findings suggested that the axillary freckles in NF-1 are simply small CAL spots and conversely, that as normal freckles show decreased melanocytes (Breathnach 1957), the CAL spots in normal individuals are just large freckles. Their patient numbers, however, were small.

In another study, Benedict *et al* (1968), studying only NF-1 sufferers, found a similar significant increase in DOPA-positive melanocytes in both CAL spots and uninvolved skin ($p=0.05$). Their study highlighted an apparently more specific feature in that the melanocytes showed large numbers of giant pigmented granules (now termed melanin macroglobules) in CAL spots; they were also present, but less frequent, in normal skin. Melanin macroglobules (MMG), however, are not specific to NF-1, being an occasional finding in normals and being seen in other disorders, including Albright's syndrome, LEOPARD syndrome and *Xeroderma pigmentosum* (Fitzpatrick and Martuza, 1986).

To assess whether MMG could be used as a diagnostic marker for NF-1, Martuza *et al* (1985) studied their number per high-power field

on light microscopy in CAL spots in NF-1 sufferers and in normal individuals. The NF-1 CAL spots had a significantly greater number ($p < 0.0008$). However, all the NF-1 patients were ≥ 16 years of age and at this age other NF-1 features are usually present. Riccardi and Eichner (1986) reported that CAL biopsy from two children with NF-1 showed only occasional MMG, suggesting the number is age-related and that MMG would not be useful as a diagnostic marker in children.

(ii) Neurofibromas - The neurofibromas in NF-1 can occur anywhere in the body, arising from sensory and autonomic nerves. It is the dermal neurofibromas that are regarded as a major defining feature. How frequently asymptomatic neurofibromas occur elsewhere in the body is an unknown factor; from the patient's viewpoint, however, it is only when they become symptomatic that problems arise, and for the purpose of the present study, symptomatic neurofibromas occurring "internally" have been regarded as complications of the disease (e.g. spinal and gastrointestinal neurofibromas).

The neurofibromas in NF-1 are indistinguishable histologically from solitary neurofibromas occurring in otherwise normal individuals (Harkin and Reed 1969). Cutaneous neurofibromas are well circumscribed but not encapsulated, the nerve of origin is not usually obvious. Neurofibromas occurring elsewhere (e.g. on nerve roots, larger peripheral nerves) are more sharply demarcated and have an apparent capsule of perineural cells. The fibres of the nerve of origin pass through the tumour and are not stretched over its surface, as is the case in schwannomas (Harkin and Reed 1969). Microscopically the tumours consist of a disordered proliferation of schwann cell elements loosely arranged in a collagenous stroma. Other cellular elements identifiable in neurofibromas are

fibroblasts, perineurial, endoneurial and mast cells (Riccardi and Eichner 1986)

Plexiform neurofibromas are distinct from other neurofibromas in that they are locally invasive (Harkin and Reed 1969; Harkin 1986), although they are composed of the same cells. The plexiform neurofibroma grows within and along the nerve, enlarging the nerve fascicles and elongating each fascicle; this growth causes the fascicles to twist on themselves, eventually creating a lesion resembling a tangle of worms. In the early stage of the lesion, hypercellular fascicles are found. As the lesion develops, there is an increase in the number of schwann cells and/or perineural cells. A few residual axons that have not been destroyed by the tumour can be found. As the lesion grows, the fascicle can either become hypocellular and myxomatous or even more cellular; the two pathologies can be found side by side in a single lesion. The skin overlying plexiform neurofibromas may be hypertrophied or hyperpigmented.

(iii) Lisch nodules - LN are melanocytic hamartomas (Perry and Font 1982) which on light microscopy are shown to be composed of a haphazard population of spindle-shaped cells with slender dendritic processes intermixed with round, plumper cells. On electron microscopy, these cells are shown to be melanocytes with many interwoven cytoplasmic processes and containing a mixture of immature and mature cells.

(D) The different forms of neurofibromatosis - As mentioned in Section 2A, one of the problems in the past has been that patients with what are now recognised to be different types of

neurofibromatosis have been lumped together under the label of "von Recklinghausen's disease". Riccardi (1982) proposed a classification which includes 7 different types of neurofibromatosis and an eighth category for cases "not otherwise specified", the definition of the different forms depending on variation in the occurrence, number and distribution of the major defining features and associated complications, particularly tumours of the nervous system. Riccardi's classification is summarised in Table 1-4.

This classification has not been widely accepted, because the type III 'mixed', type IV variant and type VII 'late onset' forms are not defined sufficiently to make their general use clinically applicable. The National Institutes of Health (NIH) Consensus Conference on neurofibromatosis addressed the issue of nomenclature and classification in July 1987. The consensus panel decided that at present there was clear evidence to distinguish NF-1 from bilateral acoustic NF (type 2, NF-2) but that there were insufficient grounds for further subclassification at the present time. The author feels that the different types which can be classified are as follows:

(i) NF-1 - The clinical features of which are summarised in Table 1-1 and compared with which all other forms are extremely rare.

(ii) NF-2 - NF-2 is also inherited as an autosomal dominant; the major features are bilateral acoustic neuromas with or without other tumours (e.g. meningiomas, schwannomas, gliomas). The confusion with NF-1 arises because patients may also have CAL spots or peripheral neurofibromas. The largest series of patients has been reviewed by Kanter *et al* (1980) who found 42% had one or more CAL spots (but none more than 5) and 19% had one or more dermal neurofibromas. Lisch

Table 1-4 Classification of the different types of neurofibromatosis proposed by Riccardi (1982)

Type	Descriptive title	Inheritance	Presence/absence of defining features of NF-1			Major clinical features
			CAL ^a	NF ^b	LNC	
NF-I	von Recklinghausen	AD	+	+	+	See Table 1-1. Numerous complications affecting all body systems
NF-II	Acoustic	AD	+	+	0	Major feature is bilateral acoustic neuromas, occasionally with meningiomas. The CAL and NF are less numerous than in NF-I.
NF-III	Mixed	AD	+	+	?	Major feature is multiple CNS tumours (acoustics, gliomas, meningiomas). Defining features may be as marked as in NF-I, more usually as in NF-II.
NF-IV	Variant	Probably AD	±	?	?	Patients may have cutaneous changes of NF-I and/or a CNS tumour; but both may be absent and are variable. No other complications.
NF-V	Segmental	?Somatic mutation	L ^d	L	L	Features of NF-I but limited to one area/segment of the body.
NF-VI	CAL spots	AD	+	-	-	CAL spots alone segregating as AD. One family cited also have pectus excavatum and dull intelligence.

NF-VII	Late onset	?	-	+	-	Patients develop neurofibromas as their only feature from third decade.
NF-NOS	Not otherwise specified					To include patients not covered by NF-I-VII.

^aCAL = CAL spots; ^bNF = peripheral neurofibromas; ^cLN = Lisch nodules; ^dL = limited distribution

nodules are not seen in NF-2. In other words, more patients than expected had minor cutaneous manifestations, and it is this that has led authors in the past to classify patients or families with this condition as having "von Recklinghausen disease" (Crowe *et al* 1956; Rodriguez & Berthrong 1966; Lee & Abbott 1969).

The recognition of NF-2 as a separate entity is important: its clinical and genetic implications are quite different from NF-1 (Huson & Thrush 1985). The concept that acoustic neuromas occur with increased frequency in NF-1 may also be erroneous and due to lack of distinction between the two conditions. Crowe and colleagues (1956) found that 10 of their patients (approximately 5%) had clinical, radiological or pathological evidence for eighth nerve disease, but in retrospect there is good evidence for an acoustic neuroma in only 4, none of whom would satisfy current criteria for the diagnosis of NF-1. Although the remaining 6 patients appear to have NF-1, none has convincing evidence for an acoustic neuroma.

Whether NF-2 can be further subdivided into a group in which only acoustic neuromas occur and another in which there are multiple other CNS tumours in addition, as suggested by Riccardi's classification, is still to be established.

(iii) Segmental neurofibromatosis - In the study of Crowe *et al* (1956) 4 cases are reported where the CAL spots and neurofibromas were limited to one segment of the body. In a 1986 review, Carey *et al* describe 3 cases and cite a further 10 cases from the literature (Miller & Sparkes 1977; Zonana & Weleber 1984; Saul & Stevenson 1984). The patient of Zonana & Weleber had facial involvement with Lisch nodules on the ipsilateral side. Crowe *et al* (1956) suggested these cases may represent somatic mutation of the NF-1 gene and

subsequent authors have agreed with this.

(iv) CAL spots only - Two families have been reported by Riccardi (1982) in which the affected individuals have multiple CAL spots as their only defining feature. In one, both the affected mother and son are of low intelligence and have mild pectus excavation; the boy is described as having questionable unilateral axillary freckling.

The importance of these families is that the diagnosis of NF-1 can not be made in young children, with no affected relatives, and in whom the only feature is multiple CAL spots.

(v) Other possible forms - In 1985 a series of papers in one issue of *The American Journal of Human Genetics* described a series of isolated cases with the features of both NF-1 and Noonans Syndrome (Allanson *et al* 1985a; Kaplan & Rosenblatt 1985; Mendez 1985; Opitz & Weaver 1985). It was proposed that these cases may represent a new syndrome which was referred to as the "Neurofibromatosis-Noonan Syndrome" (NFNS). Since that time two reports of apparent vertical transmission of NFNS have been published (Quattrin *et al* 1987; Abuelo & Meryash, 1988).

On review of the presented cases, the present author finds it difficult to accept a new 'syndrome' with features of NF-1 and Noonans combined and no unique features. More plausible explanations are that the two genes may be closely linked or that individuals with NF-1 may occasionally have the Noonan phenotype, as has been suggested by Meinecke (1987).

(E) Differential diagnosis - In the introduction to their classic monograph, Crowe *et al* (1956) state:

"There is much in the literature which suggests the diagnosis of Neurofibromatosis is frequently beclouded with uncertainty. Our

experience would indicate otherwise."

This seems to have been the experience in all major surveys, and although some have not clearly distinguished other forms of neurofibromatosis, distinction from unrelated diseases seems rarely to be a problem.

The two other syndromes in which there is CAL pigmentation similar to that seen in NF-1 are:

(i) McCune-Albright syndrome - in which the patients have areas of CAL pigmentation, but usually with more irregular borders than in NF-1, and occurring with polycystic fibrous dysplasia and sexual precocity (McCune 1936; Albright *et al* 1937). The reported cases have been sporadic and the aetiology is undetermined (Smith 1982).

(ii) Watson syndrome - in which Watson (1967) has described the affected individuals as having multiple CAL spots alone, dull intelligence and pulmonary stenosis. Some individuals also had axillary freckling. Inheritance was autosomal dominant. These families may show overlap with those of Riccardi (1981b) with CAL spots alone and possibly with the NFNS, although despite the occurrence of pulmonary stenosis, none of the Watson syndrome patients had the Noonan phenotype (Watson 1986, personal communication).

The Proteus Syndrome, first described by Cohen & Hayden in 1979, has also been confused with NF-1 in the past. The cardinal manifestations of the Proteus Syndrome are hemihypertrophy, macrodactyly, exostoses, scoliosis, cavernous hemangiomas, lipomas and linear sebaceous nevi (Clark *et al* 1987). The hemihypertrophy,

with associated subcutaneous swelling, can resemble a large plexiform neurofibroma therefore causing confusion with NF-1, although the abnormalities in skin pigmentation in the two disorders are quite distinct. The famous Elephant Man, Joseph Merrick (Treves 1885), was believed to have NF-1 for many years, the diagnosis having been originally suggested by Parkes Weber in 1909; recently, however, Tibbles & Cohen (1986) have suggested, quite rightly in the author's opinion, that Merrick in fact had Proteus Syndrome

(F) Pathogenesis

(i) Principal theories - The pathogenesis of NF-1 is unknown, any theory has to allow for the numerous complications and the very varied gene expression. All of the previous large studies have found variable gene expression even within families; identical twins have been described which are both discordant for complications (facial plexiform neurofibromas, Diekman *et al* 1967; optic glioma, Vaughn *et al* 1981) and concordant (optic glioma, Crawford & Buckler 1983). There also appears to be a hormonal influence on the disease; the neurofibromas themselves do not usually appear until puberty and pregnancy has been reported to cause both an increase in size and number of neurofibromas (Ansari & Nagamani 1976; Swapp & Main 1973).

The three main theories of pathogenesis proposed to date have been:

(a) NF-1 as a neurocristopathy - Bolande (1974, 1981) proposed that various dysgenetic hamartomatous and neoplastic conditions were pathogenetically united by their origin in neural crest development. The disease states resulting from abnormal migration, growth or cytodifferentiation of primitive neural crest cells at various stages

of human development. Bolande used the term neurocristopathies to describe the group of conditions, and divided them into two:

1. Simple neurocristopathies - characterised by a singular pathological process e.g. Hirschsprung disease (dysgenetic) and phaeochromocytoma (neoplastic).

2. Complex neurocristopathies - syndromes of multiple and variegated simple neurocristopathies, e.g. NF-1, Sipple syndrome, neurocutaneous melanosis.

The major features of NF-1 (CAL spots, neurofibromas, Lisch nodules) all arise in tissues of neural crest origin, as do some of the complications (craniofacial abnormalities, endocrine tumours). More difficult to explain are the CNS abnormalities (neural tube origin) and those complications arising in tissue of mesodermal origin (skeletal and vascular abnormalities). The consideration of a primary neural crest problem with secondary changes in other tissues could explain this; to date there has been no laboratory evidence to prove that NF-1 is a neurocristopathy.

(b) The NF-1 mutation involves a component of cell secretory

membrane - This theory was put forward by Riccardi (1982) and proposes an actual site for the abnormality in neural crest cells in NF-1. In two earlier papers (Riccardi 1979, 1981b) he had proposed that although neural crest derived cells are probably the site of the primary defect in NF-1, the mutation is only expressed when the intracellular defect of the mutant cells had not been masked by cross-feeding from adjacent non-mutant cells, thus explaining the variation of expression of the features of NF-1.

Riccardi (1982) then proposed that the NF-1 mutation involves a hypothetical component of secretory and/or plasma membranes which he names the parareceptor component (pR); the function of the pR is in modulating the behaviour of cell receptors on the plasma membrane.

The pR may be synthesised purely for this purpose and incorporated directly into the plasma membrane, but Riccardi favours the idea that it is a component of the membrane of secretory vesicles and incorporated into the plasma membrane at the time of extrusion of vesicle contents through the plasma membrane. In cells with large amounts of secretory activity, such as neural crest cells, the influence of pR on receptor function may be a major one. If the NF-1 gene involves a pR mutation then this would result in aberrant function of multiple receptor sites (e.g. for growth factors, melanocyte stimulating hormone). In addition, adjacent cells may dampen the pR effect by decreasing secretory activity of the neural crest-derived cell or increase it by enhancing this activity or stimulating proliferation of neural crest cells.

Although this hypothesis would explain many of the phenotypic manifestations of NF-1, again there is to date no laboratory evidence to confirm or refute it.

(c) Nerve Growth Factor (NGF) - NGF is a polypeptide necessary for the growth and maintenance of sympathetic and certain sensory neurons. Early studies in small numbers of patients (reviewed by Eldridge 1981) suggested that there may be increased NGF activity in the serum of patients with NF-1 and NF-2; in each form the activity was detected in different assay systems and it was proposed that the two forms may result from different abnormalities of NGF (Fabricant *et al* 1979).

This theory has now been disproved in two ways. Riopelle *et al* (1984) investigated NGF activity in a much larger number of NF-1 patients, with an improved assay system, and found no abnormalities. Darby *et al* (1985) used the β -NGF DNA probe in linkage studies and

found multiple cross-overs, thereby excluding NGF as a 'candidate' gene for NF-1 (discussed in more detail in Chapter 2-2).

(ii) Animal models - The occurrence of single or multiple neurofibromas has been reported in various birds (Bossart 1983) and mammals including cows, horses and dogs (Luginbuhl *et al* 1968; Goedegebuure 1975; Canfield 1978). No other features similar to NF-1 however have been noted and none are apparently genetic in aetiology.

The only animal known to have abnormalities of pigmentation and to develop neurofibromas is the bicolour damsel fish (Schmale *et al* 1986). As these fish reach maturity they develop areas of thickened, hyperpigmented epithelium which is followed by the development of nerve cell tumours (neurofibromas, schwannomas and neurofibrosarcomas have all been documented). Epidemiological studies of these fish suggest an infectious rather than genetic aetiology. Schmale *et al* suggest a viral oncogene as a possible candidate.

On this note, the recent finding of Hinrichs *et al* (1987) that transgenic mice arising from the incorporation of human T-lymphotropic virus type 1 develop neurofibromas is of interest. The authors speculate that at least some cases of apparent new mutations of the NF-1 gene may have an infective aetiology.

(iii) The 'double-hit' hypothesis of Knudson - In 1971, using retinoblastoma as a model, Knudson proposed that tumours which occur both in isolated and familial forms arise from two mutational events. In the hereditary form one mutation is inherited via the germ cells and the second occurs in somatic cells. In the isolated tumours both mutations occur in somatic cells. The development of DNA markers meant this hypothesis could be directly tested at the molecular

level; the hypothesis was proved to be correct for retinoblastoma (Dryja *et al* 1984).

The complex NF-1 phenotype and the fact that neurofibromas are multicellular in origin (Fialkow *et al* 1971) means that this model is unlikely to solely account for the pathogenesis of NF-1, although clearly second-step mutations may account for some of the complications (e.g. neurofibrosarcoma, pheochromocytoma). In NF-2, however, the clinical picture would fit well for this mechanism. During the course of the present study (and including material from the one case of NF-2 identified by the study), Seizinger *et al* (1986a, 1987a) showed that this was the case. They looked for loss of heterozygosity in tumours from patients with both isolated acoustic neuroma and NF-2. They particularly focused on chromosome 22 because of previous reports of loss of chromosome 22 in cytogenetic investigation of meningiomas. Loss of heterozygosity of chromosome 22 DNA markers was shown in the acoustic neuromas and in other tumours from NF-2 patients, but not for markers on 11 other chromosomes. That the locus for NF-2 was on chromosome 22, and not a secondary event, was subsequently confirmed by linkage studies (Rouleau *et al* 1987).

(iv) "Reverse genetics" - The preceding account of the pathogenesis of NF-1 has highlighted our lack of knowledge of the underlying defect. The advent of recombinant DNA technology has given the methodology by which the gene for a particular disorder can be isolated with no prior knowledge of the underlying biochemical defect. This process has become known as 'reverse' genetics (Ruddle 1984; Orkin 1986). NF-1 is an ideal candidate for this approach. The mapping of the NF-1 gene, the primary step in the reverse

genetics procedure, was one of the primary aims of the present study; the theoretical background and approaches used are presented in Part 2 of this thesis.

3. Aims of the present study

The aims of the study described in subsequent chapters were to:

(A) Define the prevalence of NF-1 in a UK population for the first time.

(B) Perform a genetic analysis of the population defined with particular reference to mutation rate, genetic fitness, paternal age effect on mutation and parental effect on severity.

(C) Assess the role of Lisch nodules as a diagnostic aid in NF-1 and to see if they occur in the normal population.

(D) Study the age of appearance of the major defining features of the disease and the frequency of complications to derive guidelines for use in genetic counselling.

(E) Assess the adequacy of the medical care received by NF-1 sufferers in the study population.

CHAPTER 2 The South East Wales population study - ascertainment
and assessment of cases

1. The study area

The study was carried out between October 1983 and February 1986 in South Glamorgan and the west part of Gwent (the county districts of Newport, Islwyn and Blaenau Gwent). The area includes the Welsh capital, Cardiff, and two other main towns, Newport and Ebbw Vale. A map of the study area is shown in Figure 2-1. On the prevalence day (1st June 1985) the population of the study area, taken from the mid-year estimates of the Office of Population Censuses and Surveys, was 668,100.

2. Patient ascertainment

Index cases were ascertained by:

- (A) contacting general practitioners and selected consultants in the study area;
- (B) reviewing the case notes of patients identified by the in-patient activity analysis of hospitals serving the study area, available from 1969, using the codes for neurofibromatosis and its recognised complications except precocious/delayed puberty, epilepsy and intellectual handicap;
- (C) reviewing the records of patients treated for scoliosis and pseudoarthrosis from 1954 at the Prince of Wales Orthopaedic Hospital, Rhydlafer, the main orthopaedic hospital in the study area.
- (D) reviewing the records of patients identified through the neuropathology register at the University Hospital of Wales (where the Regional Neurosurgical Unit is situated) as having had a



FIGURE 2-1. Map of the study area.

tumour which could be associated with NF-1 or NF-2 removed since 1972;

- (E) reviewing the records of patients identified through the dermatology histology index at the University Hospital of Wales as having had a neurofibroma removed since 1972;
- (F) reviewing the records of the Department of Medical Genetics at the University Hospital of Wales also available from 1972;
- (G) after index cases identified by the above methods had been contacted, an article about the study was published in local newspapers requesting contact with sufferers who had not yet been identified.

After obtaining permission from their General Practitioners, the index cases were contacted by letter and those who agreed to take part were assessed clinically on a domiciliary or out-patient basis by the author. If the diagnosis of NF-1 was confirmed, a family study was undertaken and all consenting first degree relatives living within the study area were examined. A minority of index cases had died prior to initiating the study; if their hospital records indicated a positive family history, relatives were contacted through the general practitioner. Details of the medical history of deceased first degree relatives were ascertained from the family, their medical records and/or death certificates.

3. Patient assessment

A full medical history was taken from the index cases and relevant family members using the questionnaire included in Appendix C. Affected individuals underwent a complete physical examination (where 'local' conditions allowed!) and the findings recorded on the

examination record at the end of the questionnaire (Appendix C). For relatives who were found to be unaffected after cutaneous and/or slit lamp examination, the only other information recorded was height and head circumference. Height was measured using a portable stadiometer (Holtain Ltd.).

The criteria used for diagnosing NF-1 were that an affected individual had to have two or more of the following:

- A. 6 or more CAL spots measuring $>1.5\text{cm}$ in diameter or $>0.5\text{ cm}$ in diameter if the patient was ≤ 14 years;
- B. axillary freckling;
- C. 2 or more dermal neurofibromas;
- D. a plexiform neurofibroma;
- E. a first degree relative with NF-1 (based on criteria A-D).

The diameters for counting CAL spots are those recommended by Crowe and Schull (1953) and Whitehouse (1966) for adults and children respectively; they enable accurate counts and reduce the risk of confusing CAL pigmentation with either lentigo or ephelis. Since accurate counting of dermal neurofibromas proved impossible, an approximate grading system was used: grade 1 = 0; grade 2 = 1-10; grade 3 = 11-100; grade 4 = 101-500 and grade 5 = >500 . The presence/absence of Lisch nodules was examined for, using a Kowa portable slit lamp which was only available for part of the study.

In assessing relatives of index cases who had <6 CAL spots and no other features, the diagnosis was considered equivocal in those aged <5 years with 1-5 CAL spots and those ≥ 5 years with 3-5 CAL spots, whereas individuals aged ≥ 5 years with one or two CAL spots were classified as normal.

Affected individuals were assigned to a severity grade for the disease. The grading system used was developed from the one described by Riccardi & Kleiner (1977), the patients being assigned to a given grade if they had any one of the disease features or complications listed for the grade:

Grade 1 (minimal) - CAL spots only, or with unobtrusive cutaneous neurofibromas;

Grade 2 (mild) - numerous cutaneous neurofibromas but without facial disfigurement; small plexiform neurofibromas with no associated problems; asymptomatic osseous lesions; learning difficulties with normal IQ;

Grade 3 (moderate) - numerous cutaneous neurofibromas with facial disfigurement; plexiform neurofibromas with modest localised hypertrophy; visceral neurofibromas; mild retardation; scoliosis or pseudoarthrosis requiring surgery; controlled epilepsy;

Grade 4 (severe) - disease complications leading to major health impairment, often requiring surgical intervention e.g. large plexiform neurofibromas with severe secondary problems, CNS tumours, malignancy, aqueduct stenosis, severe mental retardation, phaeochromocytoma and renal artery stenosis.

4. Analysis of results

When the study questionnaire was originally designed all the recorded information was going to be stored on a computer file. As some of the questions proved to be unfruitful, a revised data input form was developed, which is included in Appendix C. The information was stored on a computer file specifically designed for the purpose by Mr G Wolak. For ease of comprehension the methods of analysis used are described with the relevant section in the subsequent chapters.

CHAPTER 3 Prevalence of NF-1 and genetic analysis of the
South East Wales Population

1. Outcome of clinical assessment of ascertained cases

Using the methods described in Chapter 2, 119 cases were ascertained who were reported to have NF-1. The outcome of follow-up of these cases is summarised in Figure 3-1. One had moved from the area prior to the study and one other could not be traced.

Twenty-four ascertained cases were excluded after examination (Table 3-1), alternative diagnoses having been made in 17 (ONF 1,4, NNF 1-15)*. The distinction from NF-1 was clear-cut in all cases. The diagnostic criteria for NF-1 were not satisfied in the remaining 7. One of these (NNF 16), aged 19 years and severely mentally retarded, had 3 large areas of atypical CAL pigmentation but no other features. One (ONF 3) had a plexiform neurofibroma of the left sciatic nerve only, and one (ONF 2) had 6 peripheral neurofibromas (2 biopsy proven) but no other abnormalities. In the remaining 4 patients (ENF 1-4), all of whom had no family history of NF-1, the only cutaneous feature was CAL spots. One of these (ENF 3) had had scoliosis surgery, but the number of CAL spots was thought to be within normal limits in this and 2 other cases. The fourth case (ENF 4), who was ten months old when seen, had 4 CAL spots >1.5 cm in diameter and 10 which were >0.5 cm; her parents were normal on examination. Although she may have NF-1, the diagnosis cannot be made until she develops

* The reference numbers given in parentheses in this paragraph refer to Appendix A where the reader can find detailed clinical descriptions of these cases.

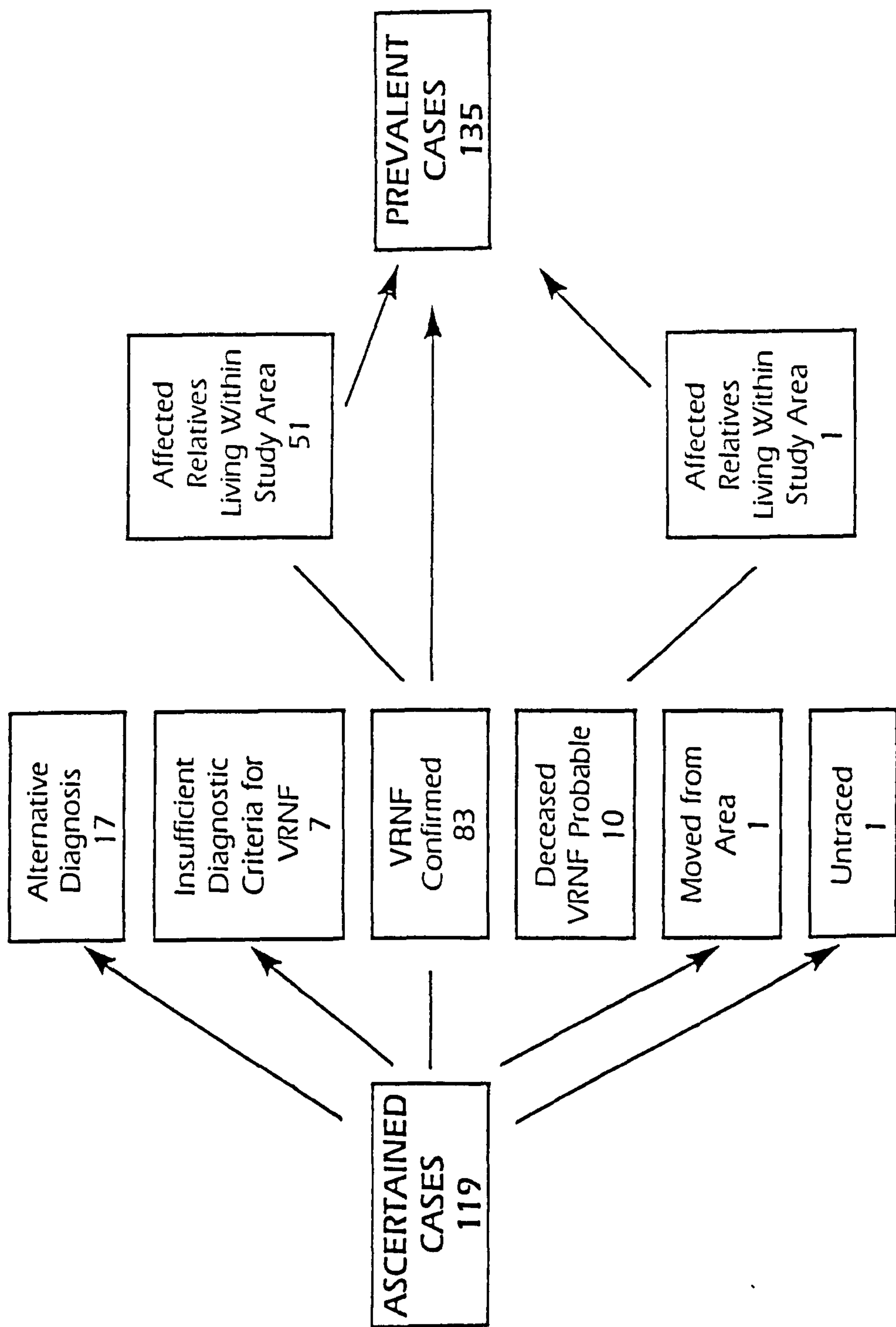


FIGURE 3-1. Outcome of follow-up of the 119 cases originally ascertained as having NF-1.

Table 3-1 Exclusions based on clinical features in 24/119 cases
originally ascertained

Excluded because of alternative diagnosis

<u>Diagnosis</u>	<u>No. of cases</u>	<u>Study Reference</u>
		<u>Number</u>
Multiple lipomas	11	NNF 1-11
Multiple moles	1	NNF 13
Steatocystoma multiplex	1	NNF 14
LEOPARD syndrome	1	NNF 15
Rheumatoid arthritis (nodular)	1	NNF 12
Multiple spinal schwannomas	1	ONF 4
Type 2 neurofibromatosis	1	ONF 1

Excluded because of insufficient diagnostic criteria

Clinical features

Mental retardation, 3 large areas of atypical CAL pigmentation (age 19)	NNF 16
Plexiform neurofibroma of left sciatic nerve only (age 50)	ONF 3
6 peripheral neurofibromas, no other features (age 43)	ONF 2
14 CAL spots >0.5 cm diameter, no other features (age 10 months)	ENF 4
4 CAL spots, no other features (age 24)	ENF 1
2 CAL spots, corrected scoliosis, no other features (age 24)	ENF 3
1 CAL spot, no other features (age 30)	ENF 2

other features. The clinical features of these 24 cases are reported in sections 2-4 of Appendix A.

Ten of the originally ascertained cases had died prior to the study, but the diagnosis of NF-1 seemed definite on reviewing their hospital records. Of these, in 3/10 instances, one or more first degree relatives had been independently ascertained (families NF 13, 18, 29); 5/10 had no relatives living in the area, and the general practitioner indicated that there were no affected individuals in one other family. In the remaining family (NF 53) one previously unrecognised affected parent was identified.

The diagnosis of NF-1 was accepted in the remaining 83 ascertained cases either as a result of examination (77/83) or on good circumstantial evidence (6/83) using existing hospital records (cases 71, 77, 80, 117, 129) or details from the general practitioner (case 135). These 83 patients came from 68 families in which a further 115 at-risk relatives were identified: NF-1 was diagnosed in 47/102 who were personally examined and 4/23 of the remainder who did not want formally to be assessed, either from their obvious facial cutaneous neurofibromas or hospital records. The remaining 64 at-risk relatives were thought not to be affected either on the basis of examination (45/64) or details from the index case, who did not want their relative to be seen (15); findings were equivocal in 4/64, aged 1 month to 6 years (NF 7, IV-5; NF 28, III-2; NF 56, IV-4; NF 61, III-4)

In summary, 69 families with one or more affected members with NF-1 were living in the study area and were identified through 84/119 of the originally ascertained cases. These 84 cases are hereafter referred to as index cases; in 13 families there were 2 or more index

cases. A total of 135 individuals (60 males and 75 females) with NF-1 were alive and resident in the study area on the prevalence day (83 index cases and 52 affected relatives). The age distribution at prevalence given as age range in years (no. of cases), was: 0-9(16), 10-19(30), 20-29(26), 30-39(20), 40-49(12), 50-59(15), 60-69(10), 70(6); the age range at time of clinical assessment was 11 months to 83 years.

The clinical summaries and pedigrees for these families are given in Section 1 of Appendix A (families NF 1-69).

2. Outcome of analysis

The results presented in this chapter are reported in the paper of Huson *et al* (1989a). The author was assisted with the statistical analyses by Dr P Clark, Section of Medical Statistics, Clinical Research Centre, Harrow.

A. Pedigree analysis - The pedigrees confirm the autosomal dominant inheritance of NF-1. There are numerous examples of male to male transmission, and segregation analysis, in families where the index case is an adult and all their offspring examined, shows no departure from the expected 1:1 ratio (23/47 children affected). There are 45 instances of 3 generation and 1 of 4 generation transmission of the disease; in all cases the intervening parent had appropriate manifestations of the disease for their age.

Forty-one index cases, 6 affected parents and 1 affected grandparent gave no history of NF-1 in their siblings or in preceeding generations. Examination of the parents and children of these cases was undertaken wherever possible. The patients were

often reluctant to involve their siblings in the study, being adamant that in all cases they were unaffected. A summary of the pedigree information for these 48 'sporadic' cases is given in Table 3-2; there was sufficient information for 41/48 to assume that they represent new mutations of the NF-1 gene. The case numbers of the 48 cases are listed in the Introduction to Section 1 of Appendix A.

Thirty-six of 41 new mutations were ≥ 20 years of age, 19 of whom had children, and in 14 of these cases, all the children were examined and classified unequivocally. Sixteen of 29 children were affected showing no significant departure from the 1:1 ratio expected for a dominant gene (using the formula given by Roberts and Pembrey, 1978; $\chi^2=0.14$, $p=0.71$).

B. Disease prevalence - On prevalence day (1st June 1985), the population of the study area, taken from the mid-year estimates of the Office of Population Censuses and Surveys, was 668,100, giving a minimum prevalence of 135/668,100, i.e. 1/4950, or $20.2/10^5$ of population.

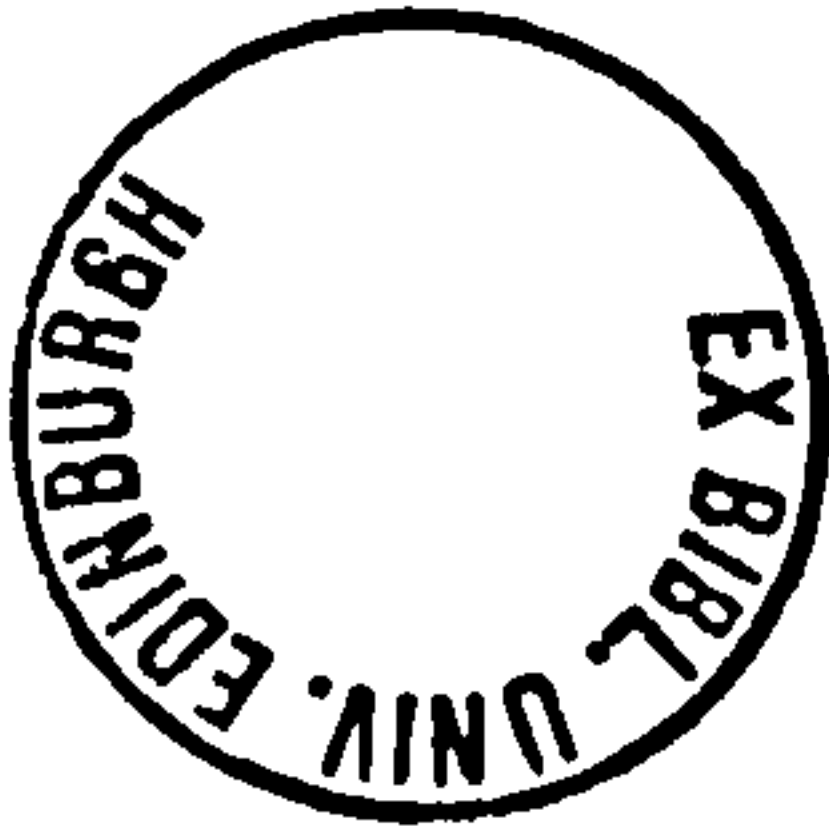
The methods of ascertainment used could, however, have been expected to 'miss' mildly affected cases, particularly children who were new mutations and had no major disease complications. This possibility was examined by comparing the ratio of new mutations to familial cases with age; this showed marked disparity. Excluding the 7 cases in whom details of inheritance were uncertain, 5/45 (11%) of cases aged <20 years were new mutations (ratio 1:8), compared with 36/83 (43%) of cases aged ≥ 20 years (ratio 1:1.3). To bring these two ratios to equality would require 26 further cases aged <20 years. If it is assumed that these 26 cases were 'missed' by the study, the

Table 3-2 Breakdown of pedigree information based on parental details for 48 cases with no history of NF-1 in preceding generations or their siblings

Parental data	No of NF-1 cases	Sibling data		Offspring data			
		Living Examined	Not examined: reported normal	Deceased	Affected	Unaffected	Equivocal Not examined: reported normal
Both parents examined	15*	4	33	1	2	5	1 0
One parent examined/ one deceased	3*	0	9	1	4	4	0 2
Parents divorced, one examined	1*	0	1	0	0	0	0 0
Both parents deceased	16	2	43	30	12	5	0 3
One parent deceased/ patient unwilling for other to be seen	5	1	8	5	5	3	0 2
Both parents refused	1	0	1	0	0	0	0 0
Total	41	7	95	37	23	17	1 7

Details inadequate: 7 - Adoption: 2; Patient confused: 2; Patient uncertain: 2; Details from GP uncertain: 1.

* In 20/34 parents, slit lamp examination was performed.



corrected prevalence is 161/668,100, i.e. 1/4150, or 24.1/10⁵ of population.

C. Estimation of fitness in NF-1 - Tanaka (1974) has shown that
Relative fitness, $w = \frac{A_p}{A_o}$

where A_p = frequency of trait among parents of index cases and A_o = frequency of trait among offspring of index cases.

In the calculation of A_p , 4 index cases (cases 40, 52, 112, 129) were excluded since parental disease status was uncertain, and in families with >1 index case, the parents of the oldest case were used. Twenty-nine of 130 parents had NF-1 ($A_p = 0.223$).

For the estimation of A_o , adult index cases where all their children had been examined and the disease status certain, were used; 18/38 were affected ($A_o = 0.474$), therefore:

$$\text{Relative fitness, } w = \frac{0.223}{0.474} = 0.470$$

Tanaka (1975) has further shown that the relative fitness in males (w') and females (w'') can be determined using the formulae:

$$w' = \frac{A_p'}{A_o'} \times \frac{x' + x''}{2x'}$$

and $w'' = \frac{A_p''}{A_o''} \times \frac{x' + x''}{2x''}$

where A_p' and A_p'' are the frequencies of the disease in fathers and mothers of index cases respectively; A_o' and A_o'' are the frequencies of affected individuals among children of male and female index cases and x' and x'' the relative frequencies of affected males and females in the general population respectively.

In the population studied, $A_p' = 11/65 = 0.169$; $A_o' = 6/10 = 0.6$; $A_p'' = 18/65 = 0.277$; $A_o'' = 12/28 = 0.429$; $x' = 60/324,100 = 0.000185$ and $x'' = 75/344000 = 0.000218$. Therefore:

$$w' = \frac{0.169}{0.6} \times \frac{0.000403}{0.000370} = 0.307$$

and $w'' = \frac{0.277}{0.429} \times \frac{0.000403}{0.000436} = 0.597$

demonstrating a large reduction in relative fitness of males compared with females.

D. Mutation rate of the NF-1 gene

(i) Direct method - Forty-one of 135 cases of NF-1 identified in the study population (668,100) represent probable new mutations of the disease gene. The mutation rate (μ , \pm 95% confidence interval; Emery 1986, p.33) is therefore equal to:

$$\mu = \frac{41}{1336200}$$

$$\mu = (3.07, \pm 0.94) \times 10^{-5}$$

In order to correct for probable under-ascertainment of children who represent new mutations, two approaches have been used:

(1) by analysing the study population aged ≥ 20 years only, 36 new mutations were identified in a population of 438,900.

$$\mu = \frac{36}{967,800} = (3.72, \pm 1.22) \times 10^{-5}$$

(2) by adding the 26 'missed' NF-1 cases, presumed to be new mutations, to the 41 actually identified:

$$\mu = \frac{67}{1336200} = (5.01, \pm 1.20) \times 10^{-5}$$

(ii) Indirect method - This calculation (Emery 1986, p.34) requires the incidence of the NF-1 gene at birth, and this is unknown.

However, given that the complications of NF-1 which cause death in childhood are rare, we have calculated the prevalence in those aged <20 years and assumed that this approximates to incidence.

Forty-six cases were ascertained aged <20 years from a population of 184,200, giving a minimum prevalence in this age group of 0.000250, or 1/4,000.

Adding the 26 paediatric cases that we have shown were 'missed' by the study methods gives a maximum prevalence of 72/184,200, 0.000391 or 1/2,558 of population. Therefore:

(a) Using minimum prevalence at <20 years as \equiv incidence

$$\mu = \frac{1}{2} I (1-f), \text{ where } I=\text{incidence and } f=\text{fitness of NF-1}$$

$$\mu = 0.000125 (0.53)$$

$$\mu = 6.62 \times 10^{-5}$$

(b) Using maximum prevalence at <20 years as \equiv incidence

$$\mu = 0.0001955 (0.53)$$

$$\mu = 10.36 \times 10^{-5}$$

Summary - The mutation rate of NF-1 therefore appears to be in the region of $3.1-10.4 \times 10^{-5}$

E. Analysis of parental age and mutation

(a) Birth-order effect - Two of 41 of the cases (5,10) classified as new mutations were the parents' only child and for 6/41 inadequate information was available for analysis of birth-order (41,44,46,74,78,135). The birth order data for the remaining 33

Table 3-3 Distribution of 33 new mutations by birth order

Birth Order											
Sibship size	1	2	3	4	5	6	7	8	9	10	Total
2	6	4									10
3	2	3	1								6
4	-	2	2	1							5
5	-	-	-	-	1						1
6	-	2	1	-	2						5
7	-	-	-	1	-	-	1				2
9	-	-	-	-	-	-	-	1	1		2
10	-	-	-	-	-	1	-	-	-	-	1
11	-	-	-	-	-	-	-	-	1	-	1
Total	8	11	4	2	3	1	1	1	2	-	33

Observed 6A = 624. Theoretical 6A = 537. Variance of 6A = 2481.

Z = $\frac{\text{Observed 6A} - \text{Theoretical 6A}}{\sqrt{2481}} = \frac{624-537}{\sqrt{2481}} = 1.747$: P=0.081

Standard Error of 6A $\sqrt{2481}$

cases are shown in Table 3-3. Using the method of Haldane and Smith (1947a) to analyse the data, no significant birth order effect is demonstrated ($p=0.08$).

(b) Parental ages of mutations - The observed ages for parents of cases classified as being new mutations were compared with expected ages using the method described by Bunday *et al* (1975). Therefore the expected maternal age, adjusted for parity, was taken directly from the Registrar General's population data and the expected paternal age calculated by adding the expected mean difference in age between spouses. Accurate parental dates of birth were known for 29/41 presumed new mutations, but maternal ages adjusted for parity are only available from 1938, therefore only 21/29 who were born after 1938 were used in analysis. The results are presented in Table 3-4. No parental age effect was demonstrated, nor was the parental age difference significantly different from expected. A further analysis of parental age was performed on 15/21 new mutations in which both parents had been examined, and no significant parental effect was found ($p=0.55$).

F. Parental effect on severity - 124/135 ascertained cases were included in this analysis; the remaining 11 cases were excluded either because of inadequate pedigree or clinical information. The severity grades for these patients were analysed using analysis of variance with parental disease status (affected father, affected mother, new mutation) and age (<18, ≥18 years) as factors. The results are shown in Table 3-5. There were no overall differences between the status categories ($p=0.84$) or between the age groups

Table 3-4 Mean parental ages of NF-1 cases classified as new mutations
and born after 1938 (n=21) compared with parental ages in the
general population

n = 21	Mean paternal age (years)	Mean maternal age (years)	Mean paternal - maternal age difference (years)
<hr/>			
Observed	29.62	27.68	1.94
Expected	29.91	27.62	2.29
<hr/>			
Mean observed- expected difference	-0.29	0.06	-0.34
Standard error of differences	1.04	0.95	0.57
<hr/>			
t*	-0.28	0.06	-0.60
p	0.79	0.95	0.55

* One sample *t* test of whether mean observed-expected difference was different from zero (df=20).

Table 3-5 Mean severity grade of 124 cases of NF-1 classified by
disease status of parents and analysis of variance
between groups

Disease status of parents		Age group		
		<18 years	≥18 years	All ages
	n	11	15	26
Affected	mean severity grade	1.73	2.53	2.19
fathers	95% CI	1.18,2.28	2.06,3.00	1.83,2.55
	n	23	36	59
Affected	mean severity grade	2.00	2.25	2.15
mothers	95% CI	1.62,2.38	1.95,2.55	1.91,2.39
	n	4	35	39
New	mean severity grade	3.25	2.23	2.33
mutations	95% CI	2.34,4.16	1.92,2.54	2.04,2.62
	n	38	86	124
All cases	mean severity grade	2.05	2.29	2.22
	95% CI	1.75,2.35	2.09,2.49	2.06,2.38

Analysis of variance

Between parental disease status groups: F=0.179, df=2,118, p=0.84

Between age groups: F=1.146, df=1,118, p=0.31

Status x age interaction: F=4,574, df=2,118, p=0.012

Mean square error = 0.8440, df=118.

($p=0.31$). However there was a significant interaction between age and status ($p=0.012$), indicating that the young new mutations tended to be more severely affected than the older new mutations; there was no age difference effect for the other status groups.

3. Discussion

In the assessment of the originally ascertained cases, the diagnostic criteria for NF-1 were easily applied and other diseases distinguished without difficulty. The disorder most frequently confused with NF-1 was multiple lipomatosis. On examination, however, the lipomas were found to be quite different from neurofibromas. As lipomas are subcutaneous lesions, the only differential is from subcutaneous neurofibromas. Lipomas were found to have a softer consistency, less well-defined margins and were not painful on palpation. Subcutaneous neurofibromas usually develop on the trunks of major peripheral nerves and pain on palpation was found to be a characteristic feature.

Seven of 119 originally ascertained cases (Table 3.1) had a feature of NF-1 on examination which was insufficient to meet the diagnostic criteria. It was felt that the 3 patients with ≤ 4 CAL spots (ENF 1-3) represent a variant of normal. Crowe and Schull (1953) found that 690/6853 (10.1%) institutionalised patients, who had no other cutaneous manifestations of NF-1, had up to 5 CAL spots; the majority (684/690) had 1-3 lesions, 4 had 4 and only 2 had 5. Burwell *et al* (1982) reported that 25.3% of 732 school children aged 4-11 years had 1-3 lesions and Kopf *et al* (1985) observed this number of CAL spots in 13.8% patients attending a private dermatology

clinic. The case aged 10 months with 14 CAL spots (ENF 4) may well have NF-1 but was excluded as this was the only abnormality on examination and her parents were normal. In case NNF 16, who had 3 areas of CAL pigmentation, all of which were >25cm in diameter, and was severely retarded, no diagnosis was made; a skin biopsy was performed to exclude chromosomal mosaicism and this was normal. The two other cases in this group may fall into the broad category of neurofibromatosis; the case with an isolated plexiform neurofibroma (ONF 3) may represent a variant of segmental neurofibromatosis and the patients with 6 peripheral neurofibromas (ONF 2) is similar to cases classified as NF VII, or late onset neurofibromatosis, by Riccardi (1982). This classification is reviewed in Chapter 1.

Pedigree analysis of the 69 families with one or more affected individuals with NF-1 confirms the autosomal dominant inheritance of NF-1. Segregation analysis of the children of adult index cases showed no significant departure from the expected 1:1 ratio for a dominant disorder. In the pedigrees studied, there was no evidence of non-penetrance of the NF-1 gene; in 46 families there is at least 3-generation transmission of the gene and all obligate heterozygotes satisfied the diagnostic criteria.

In a recent paper Riccardi and Lewis (1988) cited a further 134 examples of three generation transmission of NF-1 with no evidence of non-penetrance. In the medical literature we can find only 3 examples of apparent non-penetrance of NF-1 (Spence *et al* 1983, 1986). It must be concluded that penetrance of the NF-1 gene is virtually 100% by the age of 5 years, when affected individuals will have developed CAL spots (see also data presented in Chapter 4).

For 41/135 cases there was sufficient evidence to assume that they represent new mutations of the NF-1 gene. There was no history of the disease in preceding generations or their siblings and segregation analysis of their offspring showed no departure from the expected 1:1 ratio. No families were identified where apparently normal parents had more than one affected child and no parents of apparently isolated cases with variant expression of the NF-1 phenotype. There have been a few such occurrences reported in the literature. Riccardi and Lewis (1988) report two cases of affected siblings with normal parents and a further two families in which one parent appeared to have limited expression of the NF-1 gene. In one, a mother of two affected sons had bilateral Lisch nodules as her only disease manifestation and in the other the father of an affected son had a cervical schwannoma and two peripheral neurofibromas. In addition, Rubenstein *et al* (1983) reported two families where parents with limited disease expression had affected children. In one, a father and daughter both appear to have segmental neurofibromatosis and in the other the father of a boy with NF-1 had 6 neurofibromas and a CAL spot on his left flank.

Germline mosaicism is the most obvious explanation for apparently normal parents having more than one affected child. The cases with limited expression in the parent may represent gonosomal mosaics; experimental work in the mouse and the available evidence in man suggests that the cells of the primitive ectoderm are multipotential (Gardiner 1983). A single mutation of the NF-1 gene at this stage of development could therefore result in limited disease manifestations and gonadal involvement. From the clinical viewpoint these cases emphasise the importance of examining both the skin and the irides of

parents and siblings of apparently isolated cases. In the light of the cases with limited features of NF-1 and affected children, it is also difficult to fully reassure individuals with apparent segmental neurofibromatosis that there is no risk of NF-1 occurring in their offspring, although with available information it is impossible to quantify this risk.

The minimum prevalence of NF-1 in the general population based on this study is 1/4950 or 20/10⁵. The true figure is almost certainly higher since children who represent new mutations of the NF-1 gene may have been under-ascertained. This figure compares with previous estimates of 1/2500-3300 in the USA (Crowe *et al* 1956), 1/7800 in the USSR (Sergeyev 1975) and 1/4600 in Sweden (Samuelsson and Axelsson 1981); these studies are summarised in Table 1-3. The USSR study relied upon detection of CAL spots in a population sample of 16 year olds undergoing pre-military examination, those with a significant number of lesions being referred for the survey. Although this method should have screened all males in this age group, irrespective of state of health, the low prevalence suggests a significant under-estimation. The prevalence figure quoted by Samuelsson and Axelsson (1981) approximates to that obtained in the present study and was estimated from a population survey using similar methods. The figure of Crowe *et al* (1956) may be an over-estimate as it is based on a summary of three approaches, each with wide margins of error and none population-based. Nevertheless, the actual birth incidence of the NF-1 gene may be as high as the disease frequency estimated by Crowe *et al* (1956), the decreased prevalence in the general population being accounted for both by under-ascertainment and increased mortality. In Chapter 5 data are presented suggesting

an increased mortality in NF-1 and this was also demonstrated by Sorenson *et al* (1986) in the 39-year follow-up study of the cases originally identified by Borberg (1951). The true incidence of NF-1 could only be estimated by following a large cohort of children from birth, but the prevalence data presented for those aged <20 years suggest it may be as frequent as 1/2558 or 39/105.

This study has confirmed that the genetic fitness of NF-1 sufferers is approximately half that of the general population (0.47), the effect being more marked in males (0.31) than females (0.60). Crowe *et al* (1956) found the overall fitness to be 0.53 with a similar relative reduction for the different sexes (males 0.41, females 0.75). This reduction of fitness cannot be explained solely by severe intellectual handicap or disease complications causing severe morbidity or mortality before adulthood, since these are infrequent complications. Crowe *et al* (1956) showed that a significant contribution to decreased fitness in NF-1 is failure of affected individuals to marry, although married individuals still showed a reduction (0.80). The reasons for this are not known but there is no evidence for pregnancy wastage, so that the explanation may be an overall reduction in fecundity.

The mutation rate of the NF-1 gene in this study has been estimated to be between $3.1-10.4 \times 10^{-5}$, confirming that the gene for NF-1 has one of the highest mutation rates yet identified in man. The estimates from previous studies range from 4.3×10^{-5} to 2.6×10^{-4} . The present study has not shown paternal age to be a significant factor in the aetiology of mutation. As reviewed in Chapter 1, three previous studies have shown conflicting results. The only study to show a highly significant paternal age effect was

that of Riccardi *et al* (1984). A possible explanation for the different results is ascertainment bias in the study of Riccardi *et al*. The cases analysed were drawn from a population referred to a special interest clinic and it may be that older patients of new mutations are more likely to seek specialist referral, resulting in a biased sample. Overall it must be concluded that if an increased parental age effect does exist for NF-1, it is small when compared with other disorders such as Achondroplasia or Apert syndrome (Risch *et al* 1987).

A possible maternal effect on severity of NF-1 was first suggested by Miller and Hall in 1978. Studying 62 cases aged <18 years, ascertained through the records of a children's orthopaedic and University hospital, they found an increased severity in children born to affected mothers compared with those having affected fathers or new mutations. Two subsequent reports (Carey *et al* 1979; Riccardi and Wald 1987) and the present study have not confirmed this effect in a combined total of 443 NF-1 sufferers. It must be concluded that there is no maternal effect on disease severity. In the present data (Table 3-5) the only group showing discordant severity were the new mutations aged <18 years, although the number in this group is small (n=4). This result is undoubtedly explained by under-ascertainment of new mutations with mild manifestations of the disease.

In summary, the population study showed that NF-1 is one of the most frequent dominant disorders, with virtually 100% penetrance and a high mutation rate. The study did not confirm paternal age as a factor underlying disease mutation or a maternal effect on disease severity.

CHAPTER 4 The major and minor features of NF-1

1. Introduction

At the outset of the study it was known that the major features of NF-1 were CAL spots, peripheral neurofibromas and Lisch nodules. In formulating diagnostic criteria for the study however, Lisch nodules were excluded as their frequency had only been assessed in one previous large study of NF-1 sufferers (Lewis and Riccardi 1981), and whether or not they occur in the general population was unknown. One aim of the study was therefore to determine whether Lisch nodules occur in individuals without NF-1. No previous study had looked specifically at the age of appearance of the major features to determine the age at which at-risk individuals, not showing these features, could be reassured they had not inherited the disease. Therefore, in addition to looking at each of the features from a clinical viewpoint, the data have also been analysed to answer this specific question.

Axillary freckling was first noted to be pathognomic of NF-1 by Crowe (1964), but that freckling occurs in other unusual sites (inguinal region, submammary region in females) has only recently been reported (Riccardi 1981a). The presence/absence of freckling in each of these areas was therefore noted on examination.

Although macrocephaly and short stature are frequently mentioned in early case reports of NF-1 sufferers, head size and height have only recently been assessed in large series of patients. Carey *et al* (1979) and Riccardi (1981a) reported that 35% and 27% respectively of affected individuals in their series had head circumferences above the 97th centile. Riccardi also reported that 16% had heights at or

below the 3rd centile. As macrocephaly and short stature appeared to be relatively frequent but asymptomatic findings in NF-1, they were classified as minor features rather than complications in the present study; measurement of height and head circumference was included in the study protocol.

The data presented in this chapter are based on the findings in all the examined affected members of NF 69, whether resident in or outside the study area, and families NF 70-73 ascertained via LINK for linkage studies. It was felt that the source of ascertainment would not bias the outcome of analysis for these disease features. Although 168 affected individuals were potentially available for assessing the major defining features, examination was either not performed or incomplete in 13 individuals. A portable slit lamp was available for only part of the study and the Lisch nodule data are therefore less complete.

The data presented in Chapters 4, 5 and 6 are reported in the papers of Huson *et al* (1988, 1989b).

2. The major defining features of NF-1

A. CAL spots and freckling - CAL spots had been the first major feature to appear in all cases. Parents had noted CAL spots for the first time at 4 years or less in all affected children and within the first year of life in 82%.

CAL spots were the only disease feature noted on examination of at-risk relatives subsequently classified as equivocal or unaffected. Four cases were classified as equivocal: 3 individuals aged 0.1, 1.8 and 3.5 years had 1, 2 and 1 lesions respectively; one 6 year old had

5 extremely pale lesions 1 cm in diameter. Seven of 50 (14%) individuals aged 5 years were classified as normal despite having 1 or 2 CAL spots.

On examination CAL spots were found to vary in diameter from 0.5 to 50 cm although the majority were under 10 cm; their contour was usually smooth but some, particularly larger ones, had irregular outlines. Their colour intensity varied with background skin pigmentation, often being very pale in young children when they were best assessed using a Woods lamp. The typical appearance of CAL spots in a young child is shown in Figure 4-1.

The variation with age in number of CAL spots and percentage with 6 or more is given in Figure 4-2 and Table 4-1. All but one of the cases aged ≤ 20 years had 6 CAL spots; one 17 year-old male had only 5 spots. From the age of 20 years, however, an increasing number of cases had less than 6 spots. In some of the older cases, the CAL spots may have been disguised by the presence of large numbers of neurofibromas. However, several older patients, with fewer neurofibromas, reported "disappearance" of CAL spots with age.

The age-related frequency of the different kinds of freckling unique to NF-1 is shown in Table 4-2. The overall frequencies were axillary (69%), inguinal (38%) and submammary (26% of females). Typical axillary freckling is shown in Figure 4-3. It is interesting to note that the frequency of freckling is also apparently age-related. It appears later than CAL spots, the youngest case with axillary freckling being 3 years; the age groups between 16-40 years showed the highest incidence of freckling but thereafter there was a decrease in the number of cases with freckles in all of the three regions.



FIGURE 4-1. Multiple CAL spots in a 6 year old male with NF-1 (case 164); these were the only cutaneous features of the disease.

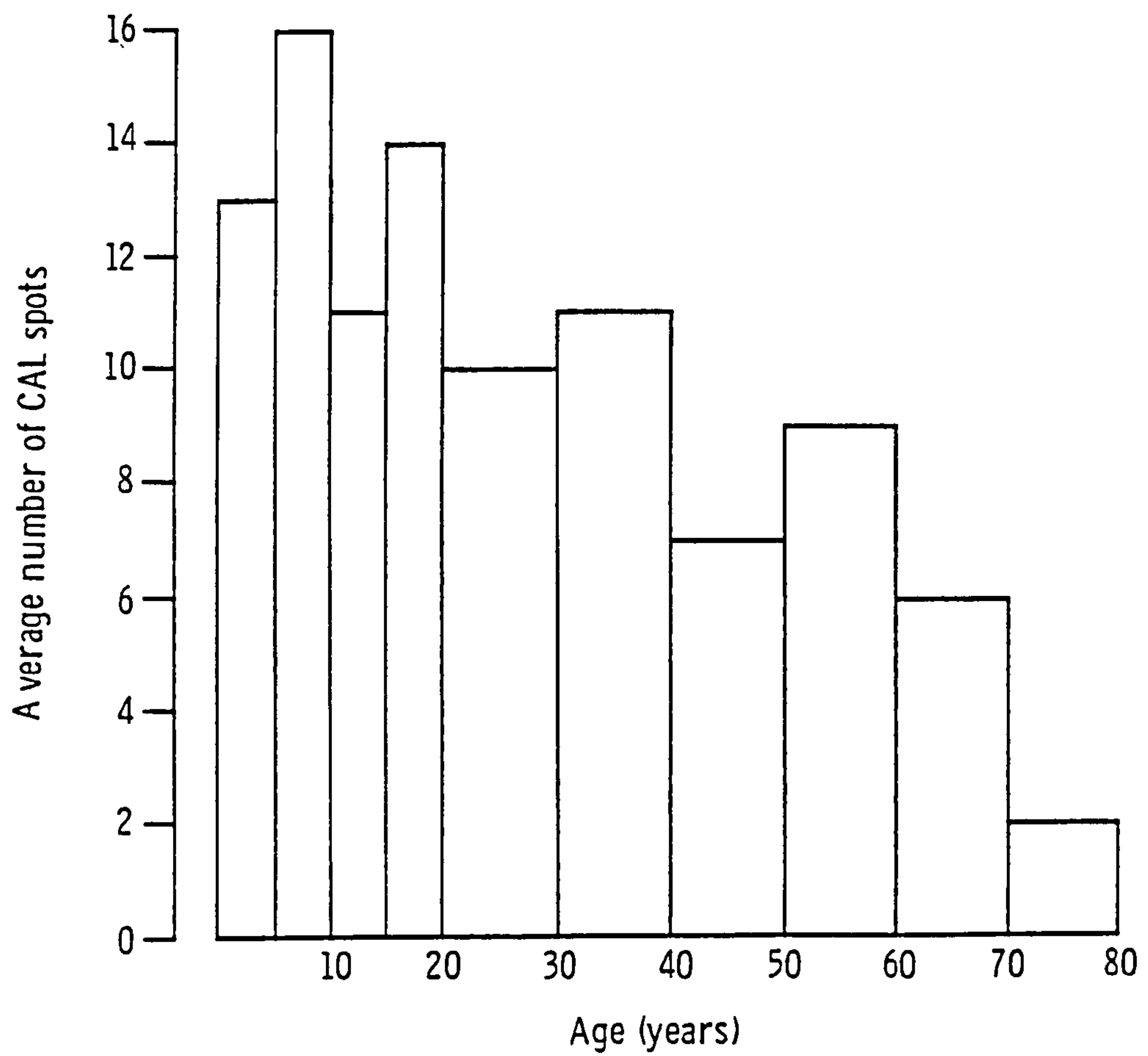


FIGURE 4-2. Variation in number of CAL spots with age (n=153).
A diameter of >0.5 cm being used for those aged
<10 years and >1.5 cm for the remainder.

Table 4-1 Age-related frequency of CAL spots (>1.5 cm diameter) and freckling

Age	No. of cases	CAL spots		Freckling		
		Min-Max no.	% with ≥6	Axillary	Inguinal	Submammary proportion
0-5	7	>0.5 cm diameter: 5-12	100	14	0	0/5
		>1.5 cm diameter: 0-9				
6-10	14	>0.5 cm diameter: 0-14	100	50	14	0/6
		>1.5 cm diameter: 6-13				
11-15	21	6-21	100	62	38	0/14
16-20	14	5-30	93	86	57	1/3(33)
21-30	28	5-17	93	75	46	5/16(31)
31-40	26	2-21	89	92	62	9/17(53)
41-50	13	3-16	62	77	46	4/11(36)
51-60	13	2-16	77	69	23	1/5(20)
61-70	13	0-13	62	62	23	0/4
71-85	6	2-5	0	67	17	0/6

Table 4-2 Percentage of cases with each of the major defining
features by age

Age	Major defining features		
	CAL spots	Cutaneous neurofibromas	Lisch nodules
0-5	100	0	66
6-10	100	14	82
11-15	100	33	71
16-20	93	93	100
21-30	93	100	90
31-40	89	100	96
41-50	62	100	88
51-60	77	100	100
61-70	62	100	88
71-85	0	100	100

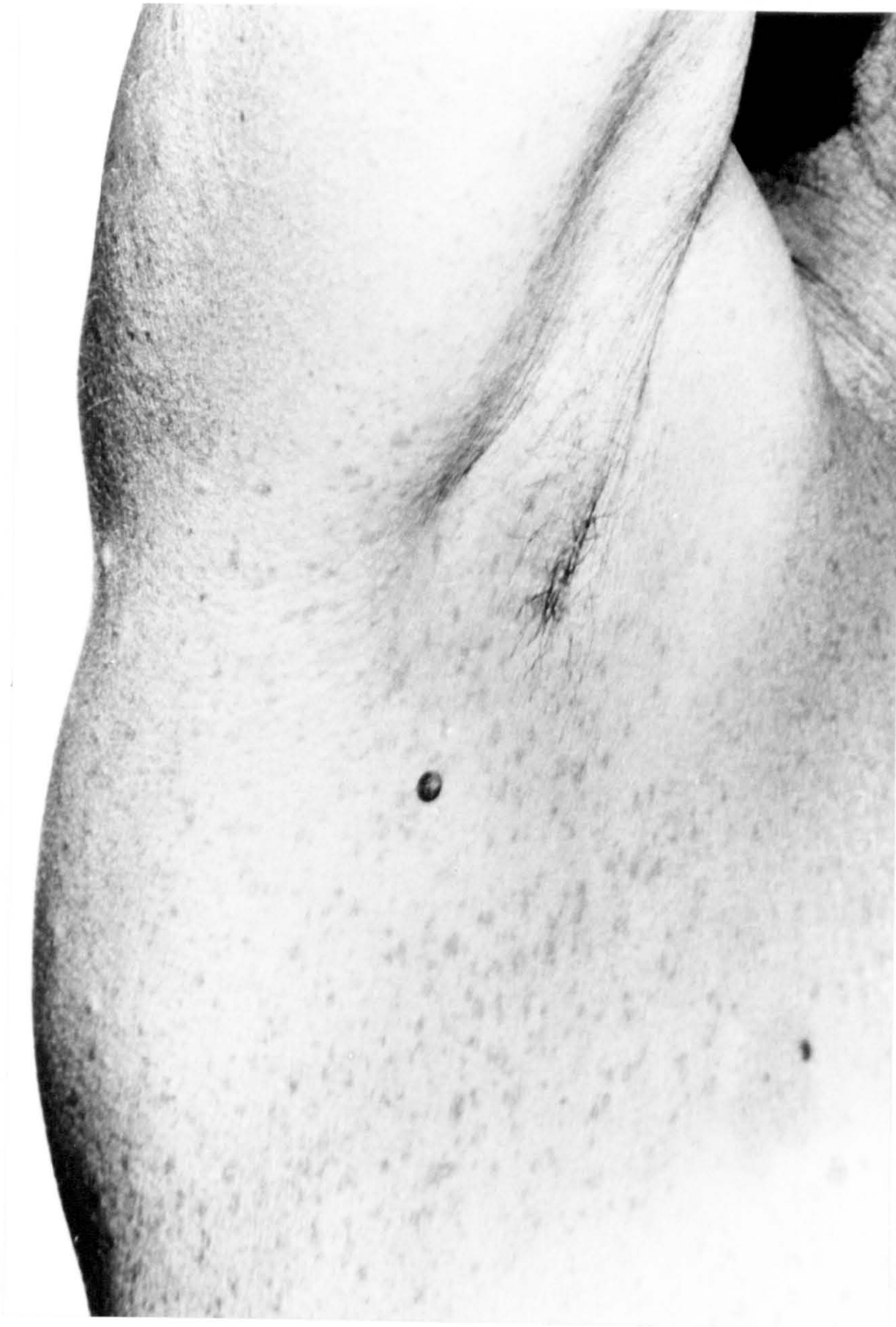


FIGURE 4-3. Axillary freckling (case 43).

B. Peripheral neurofibromas - Peripheral neurofibromas were classified as cutaneous or subcutaneous.

(i) Cutaneous neurofibromas - The cutaneous lesions lay within the dermis and epidermis moving passively with the skin and were present in all cases with peripheral neurofibromas. The majority appeared as discrete nodules with a characteristic violaceous colour and were soft, almost gelatinous on palpation, varying from 0.1 to several cm in diameter. In older patients and in all those with very numerous lesions, some had become papillomatous and were larger in size; their typical appearance is shown in Figure 4-4. The cutaneous neurofibromas were found mainly on the trunk and were only present in large numbers on exposed areas of the body in more severe cases; in only 18% of cases were there obvious lesions on the head and neck. Figure 4-5 shows the variation in number of cutaneous neurofibromas with age. The youngest patient in whom they were seen was 7 years old. The number of neurofibromas is only roughly proportional to age, severely and mildly affected cases being seen in all age groups. Although not formally tested, the number of neurofibromas seemed to show no intrafamilial correlation.

Cutaneous neurofibromas were the only one of the major disease features to cause symptoms or morbidity. These were only assessed in the 94 individuals within the population study area who had cutaneous neurofibromas. The only significant symptom was pruritis (20/94, 21%); they were rarely painful. The majority of patients had come to terms with the appearance of these lesions but 19 individuals (15 female, 4 male) were persistently distressed by their appearance. An increase in number and size of cutaneous neurofibromas during

(A)



(B)



(C)

FIGURE 4-4.

The development of the cutaneous features with age. A is a 14 year old girl with CAL spots only (case 103); B is her 42 year old father (case 97), in whom both CAL spots and neurofibromas are seen, and C is her 74 year old grandmother (case 95), with multiple neurofibromas, many of which are pedunculated.

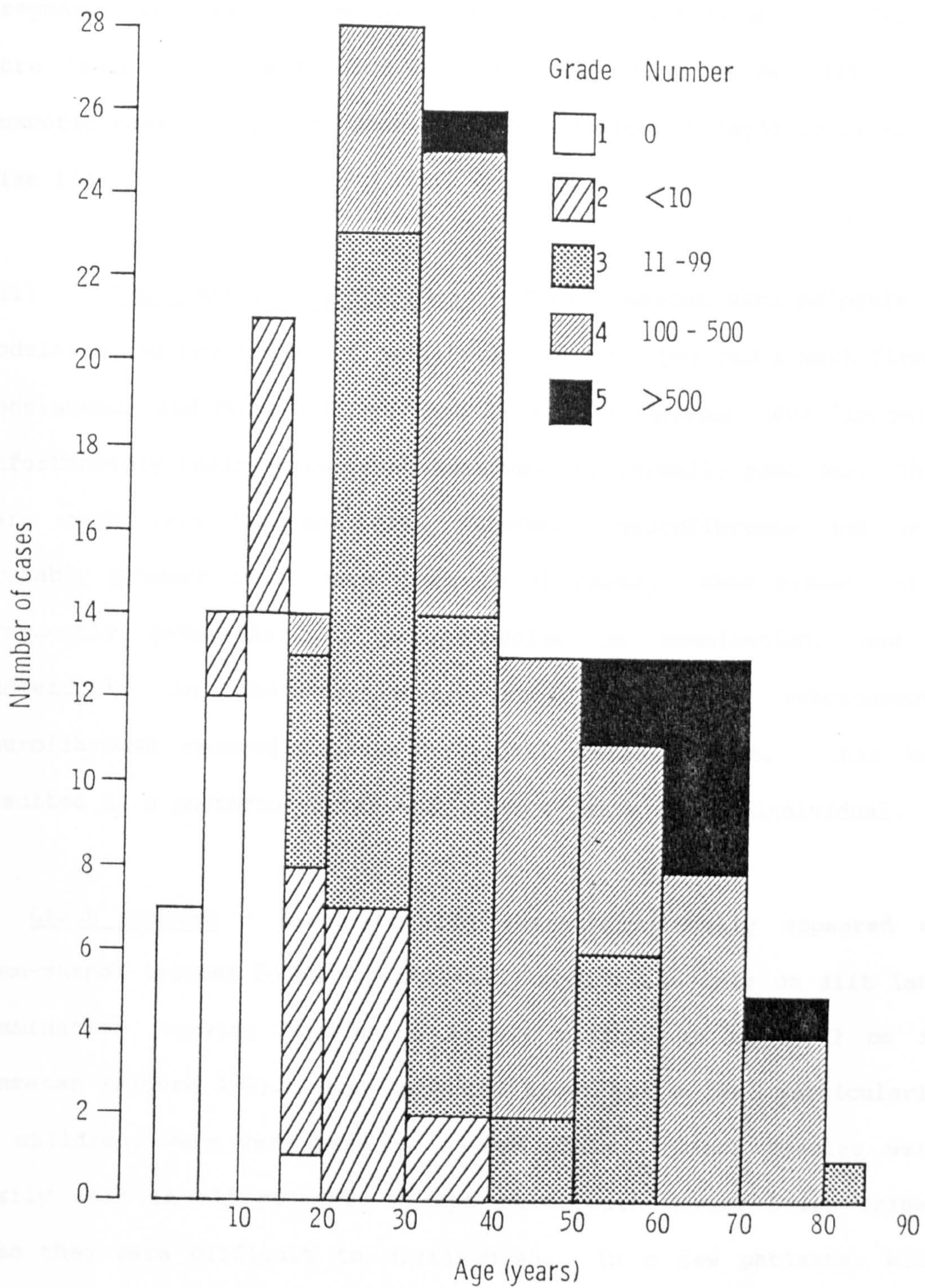


FIGURE 4-5. The comparison of number of cutaneous neurofibromas with age (n=155).

pregnancy was commented on by 10/30 (33%) of parous women. One or more lesions had been removed surgically from 41/94 (44%), for cosmetic reasons (32), diagnosis (8) or because of rapid increase in size (1).

(ii) Subcutaneous neurofibromas - These lesions were palpable as nodules along the trunks of peripheral nerves; they had a much firmer consistency and more defined margins than cutaneous neurofibromas. Unfortunately their presence/absence was not formally recorded; they were much less frequent than cutaneous neurofibromas and were probably present in no more than 5% of cases. When present they frequently gave rise to paraesthesiae on examination, and 4 individuals in the population study had had subcutaneous neurofibromas removed because of local paraesthesiae; this had resulted in a posterior interosseus nerve palsy in one individual.

C. Lisch nodules - Lisch nodules characteristically appeared as dome-shaped lesions found superficially around the iris on slit lamp examination, varying from being barely visible to up to 2 mm in diameter (Figure 4-6). Most appeared brown but a few, particularly in children, were very pale or almost white. Lisch nodules were easily seen in all patients except those with darker brown irides when they were difficult to distinguish. In a few patients, with very numerous nodules, they were visible on naked eye examination. They were easily distinguished from iris naevi, present in approximately 50% of normal adults (Michelson and Shields, 1977), by their smooth outline and shape.

The variation in presence/absence of Lisch nodules with age is

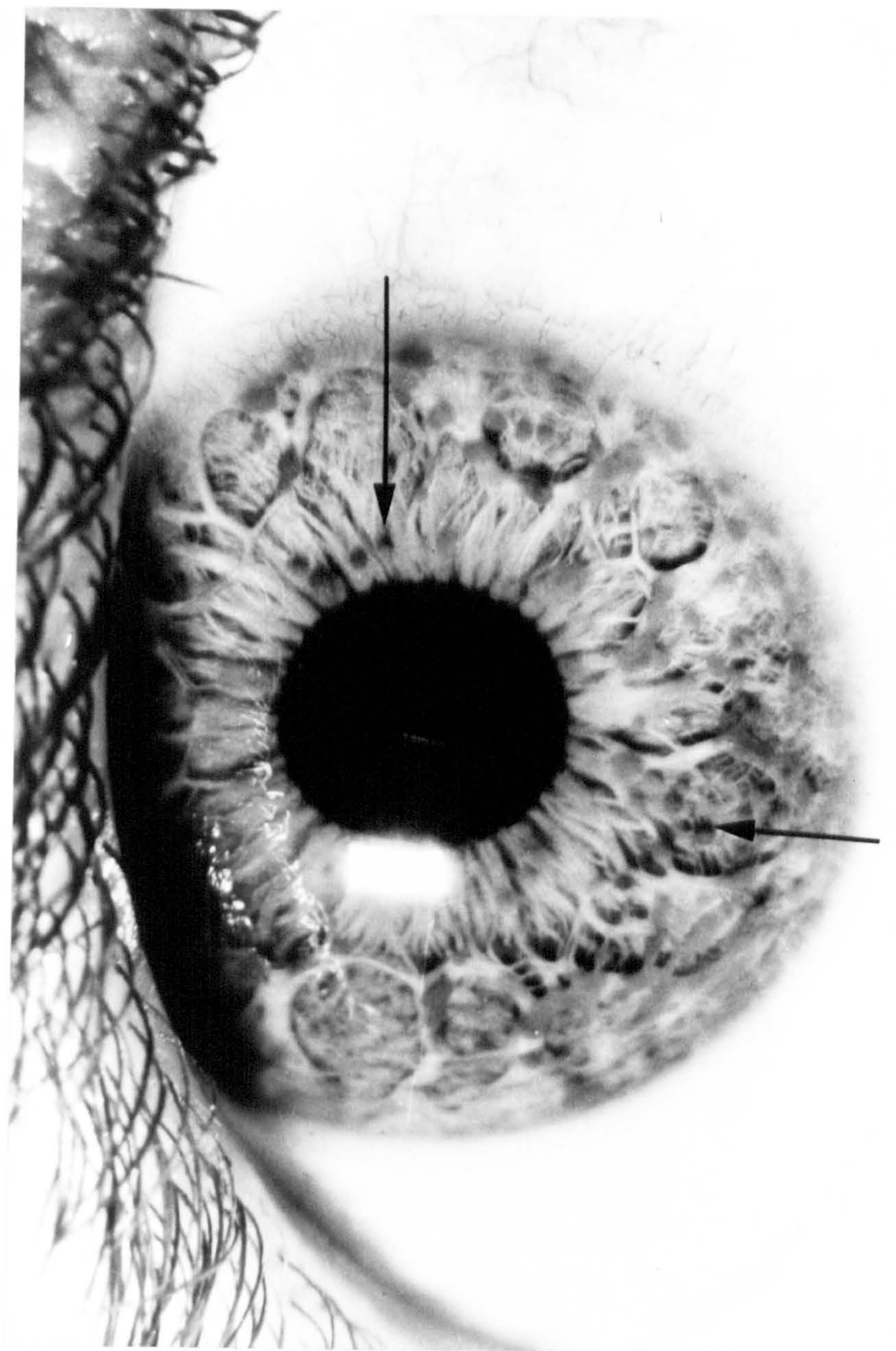


FIGURE 4-6. Lisch Nodules (case 37).

shown in Figure 4-7. They were present in 104/122 (85%) individuals overall and 71/76 (93%) of those >20 years. The youngest child in which they were seen was 3 years old, but the majority of children under 5 were unable to co-operate with examination. In 4 children Lisch nodules were only seen unilaterally.

To confirm the portable slit lamp findings a subset of patients (n=53) attended the University Hospital of Wales for examination by Mrs L Beck and Mr D Jones in the Department of Ophthalmology. Lisch nodule status of each individual was confirmed in all cases by fixed slit lamp examination and the number of nodules was counted. The mean number was 25/eye (range 1 to 100); there was a highly significant correlation between age and number of Lisch nodules/eye ($p=0.002$ using Spearman rank correlation test). These findings were reported in the paper of Huson *et al* (1987).

Mrs L Beck, in addition, examined the irides of 150 general ophthalmology outpatients to assess the frequency of Lisch nodules in the general population. No Lisch nodules were seen in 149 patients. One patient had several iris naevi and two lesions indistinguishable from Lisch nodules on the periphery of one iris. She was 27 years of age and had no family history of NF-1. Clinical examination of the patient showed no other NF-1 features; her parents had no features on either cutaneous or slit lamp examination. It was felt unlikely that this patient had 2 Lisch nodules as her sole manifestation of NF-1; the more likely explanation is that normal individuals may rarely have one or two Lisch nodules but that numbers greater than this are unique to NF-1.

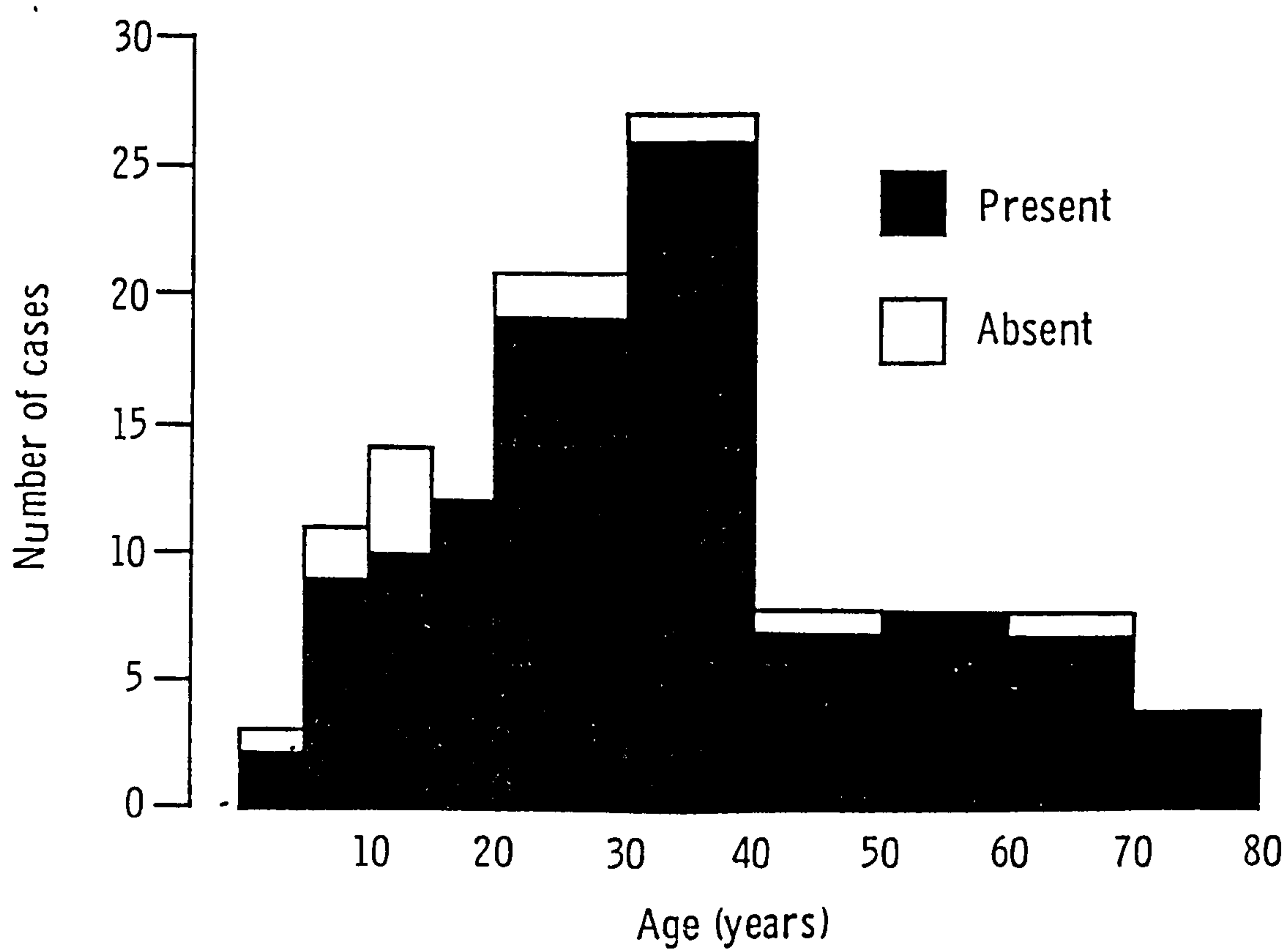


FIGURE 4-7. The variation in presence/absence of Lisch nodules with age (n=122).

D. The age of appearance of the major defining features - Two approaches have been used to look at this. Table 4-2 shows the percentage by age of affected individuals who had each diagnostic feature. In Figure 4-8 children of affected individuals, in families where the index case was an adult, have been analysed to see at what age, taking each feature in isolation, the expected 1:1 segregation ratio for a dominant disorder is reached. CAL spots are the first disease feature to appear; the data presented indicate that affected and unaffected individuals can be distinguished on this criterion alone by 5 years. Lisch nodules are the next feature to appear and it would seem that if they are going to develop, they will have done so by the mid-teens. Cutaneous neurofibromas begin to appear from the early teens but the 1:1 ratio of affected to unaffected was only reached by 20 years.

E. Discussion - The findings presented suggest that all those who have inherited the gene satisfy the diagnostic criterion of ≥ 6 CAL spots by the age of 5 years and that a large proportion do so by the age of 1 year. In the great majority of cases the distinction between normal and affected siblings was clear-cut from an early age. The only exceptions were the 4 children aged ≤ 6 years who had between 1-5 CAL spots. In view of the previous general population studies reviewed in Chapter 3, which have shown that between 13.8 and 25.3% have 1-3 CAL spots, it is likely that 3 of these children (each with 1-2 lesions) are normal. Only one case, the 6 year old with 5x1 cm diameter lesions and no other features, probably represented a truly "equivocal" diagnosis.

During the course of the study two other centres reported on the

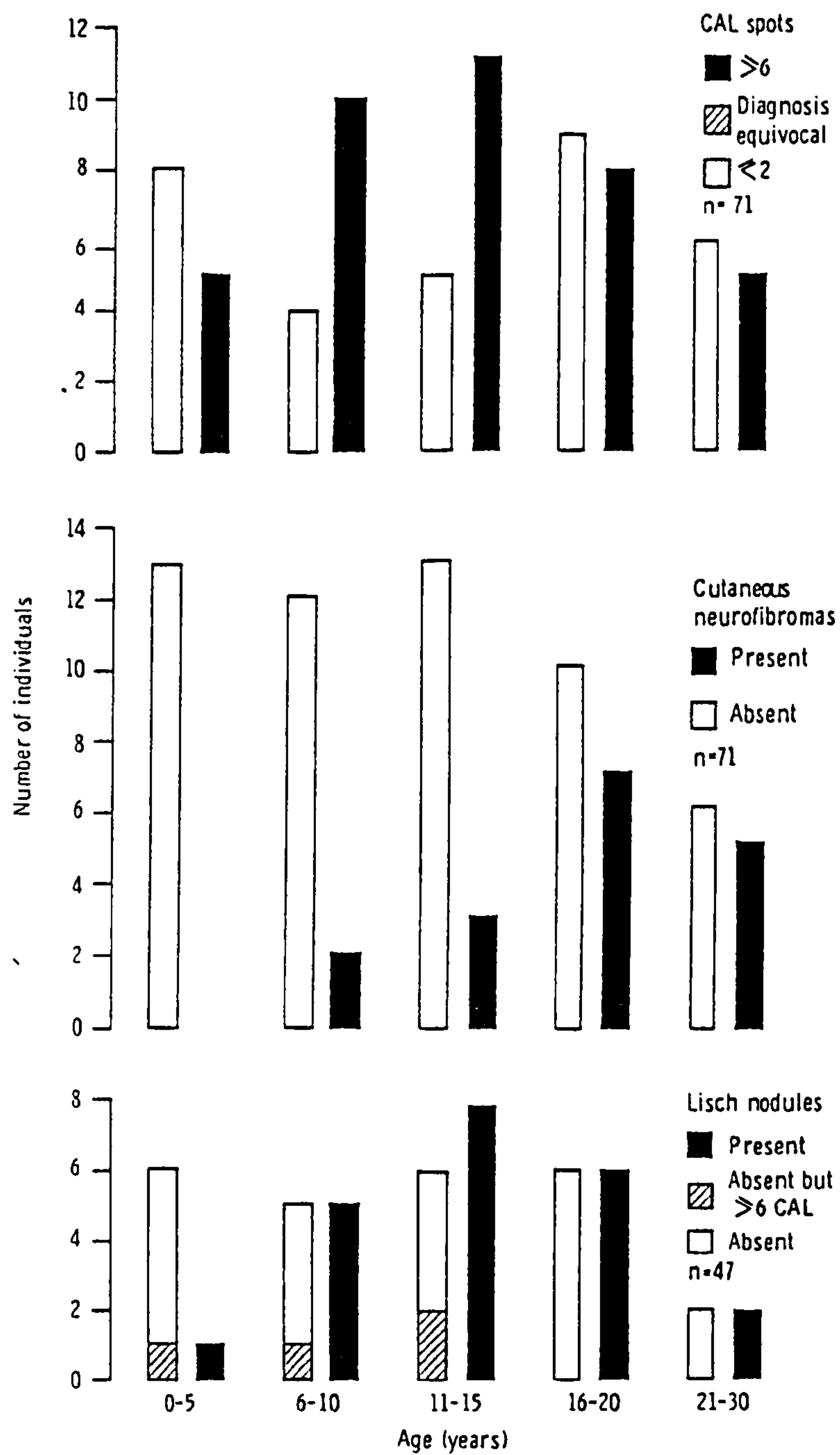


FIGURE 4-8. Presence/absence of major defining features in the offspring of affected individuals analysed by age; families in which the only index case was a child have been excluded.

frequency of Lisch nodules in NF-1 sufferers (Flueller and Boltshauser 1986; Zehavi *et al* 1986) with similar findings. Combining their data with those of Lewis and Riccardi (1981) and the data presented here show an overall frequency of 267/313 (85%) and for those aged ≥ 11 years of 229/245 (93.5%). The data presented here also suggest that even 1 or 2 Lisch nodules are extremely rare findings in the general population and therefore the finding of multiple nodules is virtually pathognomic of NF-1. Lisch nodules were included as one of the diagnostic criteria for NF-1 decided at the NIH Consensus Conference on Neurofibromatosis (1988) and since they appear before cutaneous neurofibromas, their presence should help to resolve diagnostic difficulties presented, for example, by a child with multiple CAL spots only, and in the assessment of unusual cases, as Lisch nodules have not been reported in the other autosomal dominant forms of NF-1.

The number of CAL spots and frequency of freckling have been found to decrease from middle age. Crowe *et al* (1956) also found this for CAL spots but did not look at the frequency of axillary freckling with age. Why the pigmentation, once developed, should "fade" with age is not apparent but the observation may provide insight into the underlying pathogenesis of the pigmentary abnormalities in NF-1. From a practical viewpoint, it means that cutaneous neurofibromas and Lisch nodules are more useful diagnostic criteria in the elderly.

3. Minor features of NF-1

A. Macrocephaly - After excluding patients with CNS tumours, aqueduct stenosis and large plexiform neurofibromas, 63/123 (51%) affected individuals had a head circumference at or above the 97th centile compared with 8/50 (16%) unaffected siblings ($p=0.001$). No correlation was found between intellectual handicap and macrocephaly in those in whom this comparison could be made ($n=108$, i.e. those resident in the study area); 12/46 (26%) patients with macrocephaly had required remedial class or special school education compared with 16/62 (26%) with normal head circumferences. This was also the case in the study of Carey et al (1979). The cause for the macrocephaly in NF-1 is unknown, although the study of Holt and Kuhns (1976) suggested that in some cases it was secondary to macroencephaly. They reviewed the skull X-rays of 52 patients of varying ages and found 44% of the patients had cranial capacities above the 95th percentile; four of these patients had normal pneumoencephalograms and cerebral angiograms.

B. Short stature - Excluding those with skeletal complications of the disease and identified unrelated causes for short stature, 39/124 (31.5%) individuals were at or below the 3rd centile for height. Dr S Herber of the Department of Paediatrics, University of Sheffield, kindly analysed the data further. The heights of affected individuals were converted to standard deviation scores, using the data of Tanner and Whitehouse (1976), by the formula

$$SDS = \frac{x - x_1}{SD}$$

where x is the patient's height, x_1 the population mean for that age,

and SD the population standard deviation. The SDS for affected individuals was -1.20 ± 1.07 and for normal siblings -0.12 ± 1.02 (n=34). This is highly significant (p=0.001) by unpaired t testing. There was no significant difference between the sexes - SDS affected males = -1.21 ± 1.27 (n=57), affected females = -1.18 ± 0.88 (n=67). These results imply that affected males are 8 cm under expected height and females 7.62 cm purely on the basis of having NF-1. Two of the patients in the population study had been fully investigated endocrinologically for short stature and no abnormalities detected.

This study therefore confirmed the findings of Riccardi (1981a) that a significant number of NF-1 sufferers have short stature. In a more recent review Riccardi and Eichner (1986) state that no underlying endocrinological abnormality has been demonstrated in their series. The aetiology of the short stature in NF-1 is therefore unknown.

1. Introduction

The morbidity and mortality caused by NF-1 are largely dictated by the occurrence of one or more of its complications. Accurate information regarding the incidence of complications and long term prognosis is essential for planning the management of NF-1 sufferers and genetic counselling. As reviewed in chapter 1 the difficulty is to distinguish true complications from chance associations. For the purpose of the present study, any condition was considered to be a complication of NF-1 if it had previously been associated with the disease in large population- or hospital-based surveys (Borberg 1951;* Crowe *et al* 1956;* Brasfield & Das Gupta 1972;* Riccardi & Kleiner 1977; Bader & Miller 1978; McKeen *et al* 1978; Carey *et al* 1979;* Samuelsson & Axelsson 1981;* Riccardi & Eichner 1986*). The frequency of major complications in the surveys marked with an asterisk are given in Table 1-2. The studies of Bader & Miller (1978) and McKeen *et al* (1978) are not surveys of NF-1 sufferers. Both arose as follow-up studies to series of case reports to see if two types of malignancy (childhood leukaemia and rhabdomyosarcoma, respectively) were true disease complications. The respective authors reviewed large series of patients with each malignancy and found a significant association with NF-1.

In this chapter the complications of NF-1 in the population based survey are presented. In addition, the other conditions which probably were coincidental findings are reviewed, and finally data are presented regarding disease mortality.

2. The complications of NF-1

The frequency of complications of NF-1 in the 135 cases resident within the study area is shown in Table 5-1. As no individuals in the study area aged ≥ 18 years had either a CNS tumour or malignancy with established disease associations, their frequency was estimated by looking at their occurrence in all affected family members ≥ 18 years on prevalence day (1.6.85) within and outside the study area and deceased affected relatives. In the introduction to Appendix A the case numbers of individuals with a given complication are listed so that the reader can obtain further clinical details from the appendix as necessary.

The frequency of complications in those ≥ 18 years (Table 5-1) is probably the most accurate overall picture as it avoids bias due to variable age of presentation of the complications. Most complications occurred in index cases, not affected relatives. However, the frequency of those complications which would not necessarily have led to hospital referral or identified through a search of hospital records (scoliosis not requiring surgery, plexiform neurofibromas, delayed puberty, epilepsy and intellectual handicap) did not differ between groups.

Complications occurred at random in most families although five showed apparent clustering of complications. In one (NF 17), the affected mother and a daughter had a gastrointestinal neurofibroma removed (but from different sites) and 2 further children (who lived outside the study area) had surgery for spinal neurofibromas. In two families there were 2 cases of scoliosis, although only 1 case in each had required surgery. In family NF 3, ascertained via a case who had required surgery for a thoracolumbar scoliosis, the affected

Table 5-1 Frequency of NF-1 complications in 135 cases ascertained through the population based study in South East Wales.

Complication	No. of Index cases n=83(unless stated)	No. of affected relatives n=52(unless stated)	No.(%) of all cases <18 years of age n=39(unless stated)	No.(%) of all cases > 18 years n=96(unless stated)
Plexiform neurofibromas				
All lesions	27/77	13/48	17/39 (43.6)	23/86 (26.7)
Large lesions of head and neck	4/77	0/48	3/39 (7.7)	1/86 (1.2)
Limb/trunk lesions assoc. with significant bone/skin hypertrophy	2/77	4/48	2/39 (5.1)	4/86 (4.7)
Education:				
special school education	9/73	4/51	1/34 (2.9)	12/90 (13.3)
remedial class education	14/73	7/51	8/34 (23.5)	13/90 (14.4)
normal education, specific learning difficulties	5/73	2/51	2/34 (5.9)	5/90 (5.6)
Epilepsy:				
no known cause	3	3	2 (5.1)	4 (4.2)
2° to disease complication	2	1	0	3 (3.1)
Hyperrhythmia	1	1	1 (2.6)	1 (1)
CNS tumours	2	0	2 (5.1)	2-3/138(1.5-2.2)*
Spinal neurofibromas	2	0	0	2 (2.1)
Acoustic neuroma	0	0	0	0
Aqueduct stenosis	2	0	0	2 (2.1)
Meningioangiomas	1	0	0	1 (1)
Malignancy with established disease association	0	0	0	4/138 (2.9)*
Scoliosis:				
requiring surgery	5	1	1	5 (5.2)
less severe	3	4	1 (2.6)	6 (6.3)
Pseudoarthrosis of tibia ± fibula:				
Resulting in non-union and eventual below knee amputation	2	1	1 (2.6)	2 (2.1)
less severe forms	1	1	1 (2.6)	1 (1)
Gastrointestinal neurofibromas	3	0	1 (2.6)	2 (2.1)
Renal artery stenosis	2	0	0	2 (2.1)
Phaeochromocytoma	1	0	0	1 (1)
Duodenal carcinoid	2	0	0	2 (2.1)
Congenital glaucoma	1	0	0	1 (1)
Lambdoidal suture defect	1	0	0	1 (1)

* see text for explanation

father and sister of the index case reported that they had mild curvature of the spine, unchanged from their teens. The father had a mild thoracic kyphoscoliosis and the sister a mild lumbar scoliosis. No old X-rays could be traced for further comparison. In Family NF 9 the index case had had surgery for a thoracic scoliosis and her father had a very mild thoracic scoliosis unchanged from his teens.

In a fourth family (NF 38) the 3 affected members (a mother and both her children) had each required special schooling (details of the father's schooling were not available). In the fifth family (NF 1) 2 children required special schooling and one other had received remedial class education; their parents and 3 normal siblings had attended normal school but did not obtain any academic qualifications. In both families it was felt that social and environmental factors could have made a significant contribution to the children's school performance.

A review of the complications of NF-1 in the study population is now given. Since some complications of NF-1 are rare, they may not occur in a population-based survey by chance. Those complications not identified in the study are briefly reviewed in the relevant section; it must be assumed their frequency in NF-1 sufferers is $\leq 1\%$.

A. Plexiform neurofibromas - The appearance of these lesions was clinically quite distinct from cutaneous neurofibromas. They presented as large subcutaneous swellings with ill-defined margins varying from lesions a few cm in diameter to those involving a whole area of the body (Figure 5-1). The consistency was soft although occasionally hypertrophied nerve trunks could be palpated within the mass. The skin overlying the lesion was abnormal in 23/45 lesions (5

individuals had 2) due to a combination of hypertrophy, CAL pigmentation or hypertrichosis. The age at which the lesions appeared was also quite different from cutaneous neurofibromas. Sixteen of the 40 cases with plexiform neurofibromas were aged <14 years; in 8 of these individuals the lesion had been noted within the first year of life and this was the case in all those with large facial lesions.

Plexiform neurofibromas were most frequent on the trunk (20/45), followed by the limbs (17/45) and head and neck (8/45). They were rarely symptomatic but their cosmetic burden was considerable; in Table 5-1 the frequency of the more severe lesions is presented. Four patients (28,36,109,116*) had large plexiform neurofibromas of the face; involvement of the orbit had led to loss of vision in 2 and in one overgrowth of the maxilla had resulted in problems with malocclusion. All 4 individuals had had a series of corrective plastic surgical procedures. A further group of 7 cases has been subdivided because of particularly large truncal lesions (70,115) associated with severe hypertrophy of the overlying skin and large lesions affecting the lower limb (26,49,57,76,132); in 4/5 of the latter group the lesions were around the ankle and overgrowth of the underlying bone had occurred. One case (57), whose lesion extended up the leg, had required epiphyseal plating at the knee to prevent further overgrowth of the long bones on the affected side.

Eleven individuals had had operations to improve the appearance of these lesions but in no case had complete removal been achieved, surgery being relatively unsuccessful because of involvement of

* Unless stated, numbers given in parentheses in this section are the case numbers of individuals with a given complication.

FIGURE 5-1. Plexiform neurofibromas.

A. Facial plexiform neurofibroma in a 4 year old girl (case 109) who had presented at 2 months of age. She had already had a number of surgical procedures.

B. Plexiform neurofibroma overlying the lumbar spine of an 8 year old boy (case 31). The lesion was asymptomatic and had first been noted on routine examination at 3 years of age.

C. Anterior and posterior views of large plexiform neurofibroma of left lower limb in 38 year old male (case 57).

(A)



(B)



(C)

major nerve trunks, excessive haemorrhage at operation, or poor delineation of the lesion from normal tissue. This is the experience in other large series (Riccardi & Eichner 1986).

In the older NF-1 literature, the descriptive designation of "elephantiasis neuromatosa" or "fibroma pendulum" is used to describe very large plexiform neurofibromas (Borberg 1951; Crowe *et al* 1956). The other point frequently raised in the literature (Breasfield & Das Gupta 1972; Riccardi & Eichner 1986) is that it is probably only plexiform neurofibromas that harbour the potential for malignant change. Although this was not seen in any of the 69 families in the population study, it had occurred in one of the originally ascertained cases who was a new mutation for NF-1 and died prior to the study.

B. Intellectual handicap - A retrospective assessment of the education deemed appropriate for each affected individual was used to give an approximate measure of intellectual handicap attributed to the disease. The outcome is shown in Table 5-1. In only 2 affected individuals were there NF-1 complications which might have accounted for these problems. In case 76, who had attended a special school, hypsarrhythmia might have caused the intellectual handicap and in case 65, who had attended a remedial class, hydrocephalus secondary to aqueduct stenosis might have been responsible.

Of the affected adults, only one was severely retarded, having an intellectual age of 2 years when seen at 33 years. Of the 11 other adults who had attended a special school, 2 were subjectively classified as moderately retarded, as they could barely read or write and, although living in the community, required considerable family

support; the remaining 9 individuals were classified as minimally retarded, since they were reasonably literate and leading independent lives. Those who required remedial class education were also classified as minimally retarded. Of the 34 children aged ≥ 5 to < 18 years) only one was attending a special school and was moderately retarded (aged 13 years at assessment); 8 were in remedial class education (classified as minimally retarded). Of the 7 individuals who reported specific learning difficulties, the main problem was or had been in reading, with normal performance in other areas. Combining the educational data on adults and children in terms of a subjective assessment of overall performance gives the following frequencies: severe retardation 1/124 (0.8%), moderate retardation 3/124 (2.4%), minimal retardation 30/124 (24.2%) and specific learning difficulties 7/124 (5.6%).

The educational placement of normal individuals with an affected parent was used for comparison. 43/45 (95%) of this group were attending or had been to a normal school and 2/45 (5%) were attending a remedial class. The educational achievements of the two groups also differed. Of the 95 affected individuals who had completed their education, 3 had achieved passes at GCE 'O' level, 1 at 'A' level and 1 higher school certificate, although none had gained university places. In the unaffected group, 22 had completed their education, 1 had achieved 'O' levels passes and 4 'A' level passes, of whom 3 had gained university places.

As the risk of intellectual handicap is often a major factor influencing reproductive decisions for a particular disorder, it is appropriate to review the findings of previous series in detail. The frequency of intellectual handicap in NF-1 has been looked at in some

detail in 4 previous series. Borberg (1951), who ascertained cases through hospital records, found that the distribution of IQ was <70 (7/71 - 10%); 70-80 (3/71 - 4%); 80-90 (45/71 - 63%) and >90 (16/71 - 23%); Carey *et al* (1979), ascertaining cases through genetic clinic records, found 11.4% of index cases and 4.2% of affected relatives had an IQ of <70; 11.4% of the remainder had learning difficulties. Samuelsson (1981), in a population study with similar methodology to our own, found that 2/71 (2/8%) adult cases had required special school education, and 30/71 (42%) remedial class education. Riccardi & Eichner (1986), studying cases referred to a special interest clinic, found that 18/201 (9%) cases had an IQ of <70 and 30% of the whole population (n=238) had learning difficulties. The present study confirms the high frequency of learning difficulties (5.6%) and minimal intellectual handicap (24.2%) but found a slightly lower frequency of moderate/severe handicap (2.4% and 0.8% respectively) than most previous studies. A more exact understanding of the intellectual burden of NF-1 will only be reached when a large series of children are followed, with appropriate psychometric examinations, from early childhood.

Rosman & Pierce (1967) have demonstrated a possible cause for the intellectual handicap in NF-1. They reviewed the histology of the cerebral hemispheres from 10 patients with the disease who had come to autopsy. Five of these patients had been mentally retarded and in all, cerebral abnormalities were found. These included gross and microscopic disorders of cortical architecture, and neuronal heterotopias in the deep cerebral white matter. In the remaining 5 cases, who were at least of average intelligence, similar but less severe changes were found. In view of these findings, the recent

report of Bognanno *et al* (1988) is of interest. In a survey of Cranial Magnetic Resonance images (MRI) of 53 patients with NF-1 and NF-2, they found small focal areas of increased signal within the brain parenchyma on T2-weighted images in 23 patients; CT and T1-weighted MR images revealed few or no abnormalities in corresponding locations. The lesions were located primarily in the basal ganglia and internal capsule with other lesions seen in the midbrain, cerebellum and subcortical white matter. The authors postulate that they represent focal areas of heterotopic or possibly dysplastic tissue. Unfortunately, the authors do not categorise the cases by type of neurofibromatosis and insufficient information is given to allow correlation with clinical findings. Further evaluation of MRI in patients with well-defined NF-1 will be important to see if there is any correlation between these lesions and complications such as intellectual handicap and epilepsy; follow-up studies will also indicate whether the MRI abnormalities indicate sites which may harbour the potential for neoplastic change.

C. Neurological complications

(i) Epilepsy - The study confirmed the increased frequency of epilepsy in NF-1 sufferers. Six individuals suffered from epileptic fits for which no cause other than NF-1 had been demonstrated on investigation; of these, 3 individuals had grand mal seizures (36,61,77), one both grand mal and absenced attacks (21), one complex partial seizures (10) and one child petit mal attacks (20). All had achieved acceptable seizure control on anticonvulsant treatment. A further 2 cases had seizures which were attributed to a specific

disease complication - meningioangiomas (24) and aqueduct stenosis (65). In another patient (57) with complex partial seizures CT demonstrated an area of probable infarction in the right frontal region, the cause of which was unknown.

Hypsarrhythmia also occurred more frequently in the study population than expected (2 cases) even though this is generally considered to be common in tuberous sclerosis but not NF-1. This association has previously been noted (Riccardi & Eichner 1986) so that hypsarrhythmia should probably be considered as having an increased frequency in NF-1. The two cases in the study presented at 0.6 (89) and 0.7 years (76) and showed response to ACTH treatment. One (89) had no further seizures or residual neurological deficit, although did go on to develop an optic glioma. The other (76) had required special school education and developed grand mal seizures at the age of 17 years.

(ii) Gliomas - Two of the individuals in the study population had optic gliomas. Case 48 had had a symptomatic chiasmal tumour treated with radiotherapy at 6 years. His vision improved after treatment and had then remained stable (assessed for the study at 12 years). Case 89 was diagnosed secondary to the study; he had been under review from the age of 3 years for unilateral optic atrophy with normal optic foramina on X-ray. The ophthalmologist was unaware of the family history of NF-1 and following assessment for the study (age 13 years) a CT scan was performed; it demonstrated changes consistent with an intra-orbital optic nerve glioma.

Within the study area, the prevalence of optic nerve gliomas was therefore 1.5% (2/135). The benign course of the tumours in these

cases is in keeping with reviews of previous larger series (Wright *et al* 1980; Illgren *et al* 1985) that have shown that optic gliomas in NF-1 sufferers follow a much more benign course than when they occur in isolation. As CT and MRI scans are being performed on more NF-1 sufferers, asymptomatic optic gliomas are being reported as relatively frequent findings. In the CT study of Lewis *et al* (1984) an overall frequency of 15% was reported but only 5.1% of lesions were symptomatic. In the MRI study of Bognanno *et al* (1988) 5/53 (9.4%) had asymptomatic optic gliomas.

Within the extended families of the population index cases, 3 individuals were identified who had developed a glioma of the central nervous system, of whom 2 had NF-1 (Table 5-2). The status of the third case remained uncertain even after reviewing her medical records. To estimate the overall frequency of glioma, all index cases and affected relatives aged ≥ 18 years on 1.6.85 within (n=95) and outside the study area (n=18, of whom 12 were examined), and deceased affected relatives (n=25) were used as denominator. The frequency of glioma was 2/138 (1.5%) or 3/139 (2.2%) depending upon whether the case in whom the diagnosis of NF-1 was uncertain had the disease; the 2 index cases with optic glioma were excluded because of age. The overall frequency of gliomas was therefore lower than in most previous studies (Table 1-3).

(iii) Spinal neurofibromas - Two individuals developed cord compression due to the presence of a spinal neurofibroma. Case 24 had a neurofibroma removed at C1 and C2 on separate occasions and Case 48 a neurofibroma removed at T3. Neither patient had residual neurological disability post-operatively, although it is of interest

Table 5-2 CNS tumours and other malignancy associated with NF-1 occurring in all affected living family members (718 years) and deceased relatives

<u>Case No.</u>	<u>Status</u>	<u>Tumour type</u>	<u>Diagnosis of NF-1</u>	<u>Age of presentation (and assessment in years)</u>	<u>Outcome</u>	<u>Combined frequency</u>
<u>CNS TUMOURS</u>						
136	Relative	Optic glioma	Definite	3 (33)	Enucleation of rt. eye and resection of rt. optic nerve. No further complications.	Excluding case 138 2/138(1.5%)
137	Deceased relative	Frontal astro-cytoma	Definite	32	Died two weeks after subtotal resection	
138	Deceased relative	Cerebelar astrocytoma	Uncertain	19	Died three weeks after subtotal resection	Including case 138 3/139(2.2%)
<u>MALIGNANCY WITH RECOGNISED ASSOCIATION</u>						
139	Deceased relative	Rhabdomyosarcoma	Definite	0.5	Inoperable tumour invading bladder, uterus and pelvic wall. Died one week after laparotomy	4/138 (2.9%)
140	Deceased relative	Rhabdomyosarcoma	Definite	2	Subtotal resection of bladder wall tumour followed by chemotherapy. Died 2 months later.	
141	Deceased relative	Neurofibrosarcoma	Definite	24	Resection of paraspinal mass at L1-2 followed by radio-therapy. Tumour recurred; died 5 months later	
142	Relative living outside study area	Low grade malignant neurilemmoma	Definite	33	Painful enlarging mass removed from lt. radial nerve. Not personally examined; no recurrence by time of assessment of family for study (4 years later)	

that both had other CNS complications: case 24 (meningoangiomatosis) and case 48 (aqueduct stenosis).

(iv) Acoustic neuromas and meningiomas - None of the patients in the study population or their extended families had had either of these tumours. As reviewed in Chapter 1, the previously reported association of acoustic neuromas with NF-1 may have been due to failure to distinguish NF-2 cases (Crowe *et al* 1956). This may also be the case for meningiomas as neither of these tumours have been reported in other recent large series (Samuelsson 1981; Riccardi & Eichner 1986).

(v) Aqueduct stenosis - Two individuals had been successfully treated for hydrocephalus due to aqueduct stenosis; case 48 had presented with difficulty in walking at the age of 4 years and case 65 with grand mal seizures aged 16 years. The frequency of this complication (2%) is the same as that in the series of Riccardi & Eichner (1986). Both polypoid growths of ependymal granulation tissue (Russell 1949) and gliosis (Laurence 1962) have been demonstrated obstructing the aqueduct in autopsied cases with this complication.

(vi) Meningioangiomatosis - Although one case (24) had this complication, it must be regarded as an extremely rare complication. Only 17 cases had been published prior to 1986 (Halper *et al*), 11 of these had NF-1.

(vii) Co-ordination problems - Attention was drawn to the fact that individuals with NF-1 often experience co-ordination problems only

after completion of the clinical assessment of the cases was completed (Riccardi & Eichner 1986). Although no specific abnormalities were detected on limited neurological examination of the affected individuals in the present study, several parents specifically stated that their affected offspring were "clumsy" in comparison to their normal siblings.

D. Skeletal complications

(i) Scoliosis - This was the most frequent skeletal complication, for which 6 individuals had required surgery during their teens, 4 (26,66,69,129) with thoracic and 2 (7,13) with thoracolumbar curvatures. In 3 cases, although surgery had stabilised the curvature, there was significant residual deformity (Figure 5-2). None had secondary neurological complications prior to or following surgery. In only one case (66) had dystrophic vertebral abnormalities been noted. A further seven individuals (5,6,9,25,108,120,133) had scoliosis which had not required surgery, in all but one of whom growth was complete when assessed for the study. Four out of 7 had only very discrete thoracic (120, 133) and lumbar (5,9) curves. In the 2 cases (5,120) for whom X-ray reports were available, these curves involved 3 vertebrae only and were associated with abnormalities of the vertebral body.

Scoliosis is therefore one of the more frequent complications of NF-1. In a large series of patients with scoliosis Winter *et al* (1979) found 1% had NF-1. The majority of the NF-1 cases (80/102) in the series of Winter *et al* (1979) had dystrophic changes of the involved vertebrae. The outcome of treatment was worse in those with

(A)

(B)

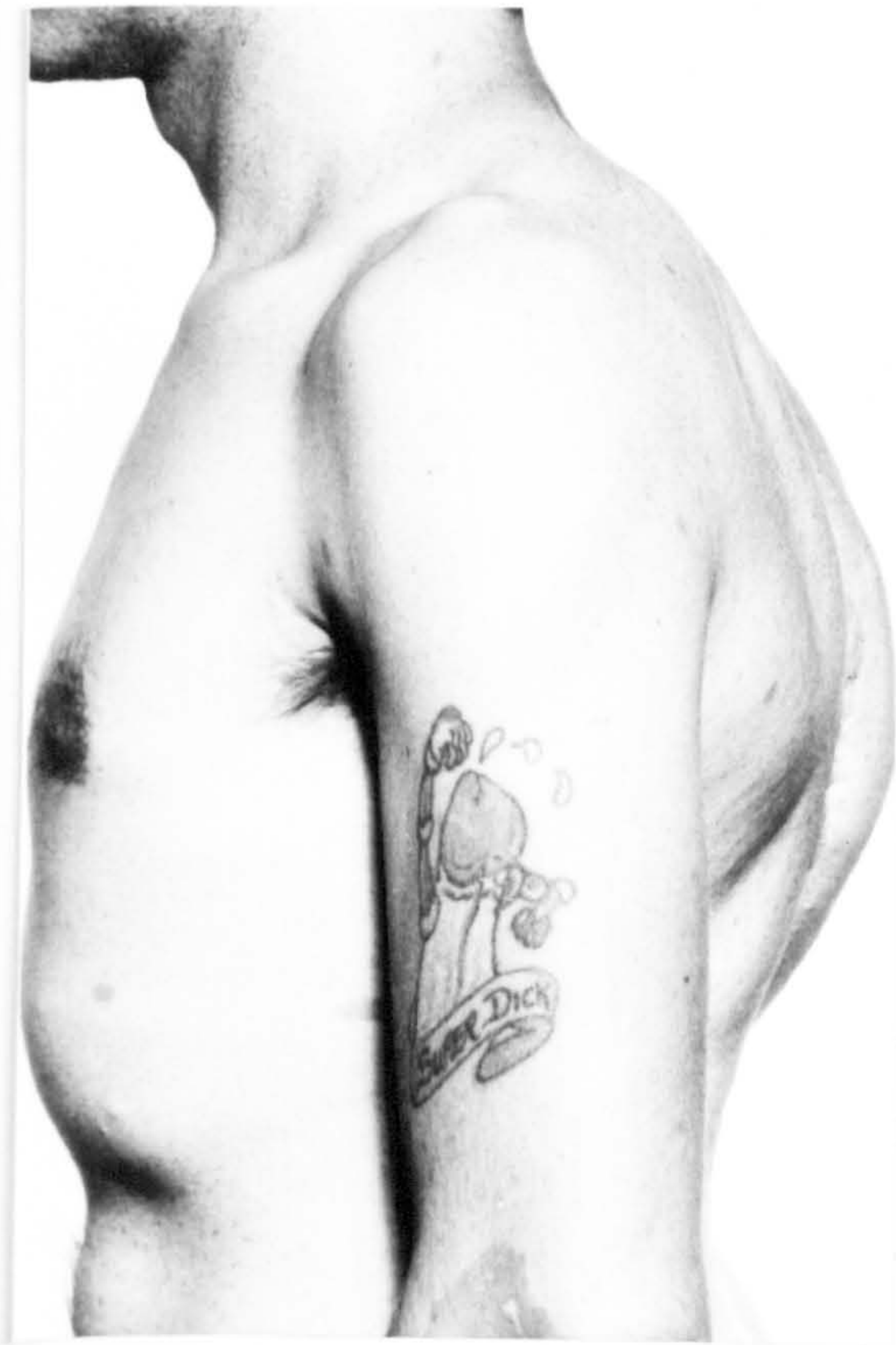


FIGURE 5-2. Scoliosis: variation in outcome of surgery.

A. Corrected scoliosis in a 26 year old male (case 69) who had no underlying vertebral abnormality.

B. Kyphoscoliosis in a 19 year old male (non study case); the involved vertebrae were abnormal and although he had had a spinal fusion (age 13 years) there is severe residual deformity.

dystrophic curves and cord compression was only seen in cases with dystrophic curves (11/102). The low frequency of abnormal vertebrae in the present series may just reflect the fact that the information was recorded from case notes and the original films not reviewed.

(ii) Pseudoarthrosis of tibia ± fibia - Four individuals had pseudoarthrosis of the tibia (17,28) or fibula (4) or both (111). The 3 cases with tibial involvement all presented within the first year of life, 2 with fractures and one with bowing which later fractured. Non-union had resulted in an eventual below-knee amputation in all 3 cases (at 11, 12 and 39 years of age). Case 4, with fibular pseudoarthrosis, presented at 14 months and was successfully treated by bone grafting. One further case (34) had bowing of the tibia and fibula, first noticed at 8 months of age but no fracture had occurred by 11 years of age when he was assessed for the study. No cases were seen of pseudoarthrosis of the long bones of the forearm which has also been reported in association with NF-1 (Gregg *et al* 1982).

Twenty of the 40 cases of tibial pseudoarthrosis in the series of Morrissey *et al* (1981) had NF-1. The underlying pathogenesis is uncertain; there are a few histologically verified cases of intraosseus neurofibromas (Green & Rudo 1943; Berk & Mankin 1964) and schwannomas (Jacobs & Fox 1972; Gordon 1976) being found at the fracture site, but these seem to be exceptional. In the larger series of patients that have been studied using both light (Aegerter 1950) and electron microscopy (Briner & Yunis 1973; Brown *et al* 1977), no underlying tumours have been found and the authors have concluded that defective bone formation is the underlying

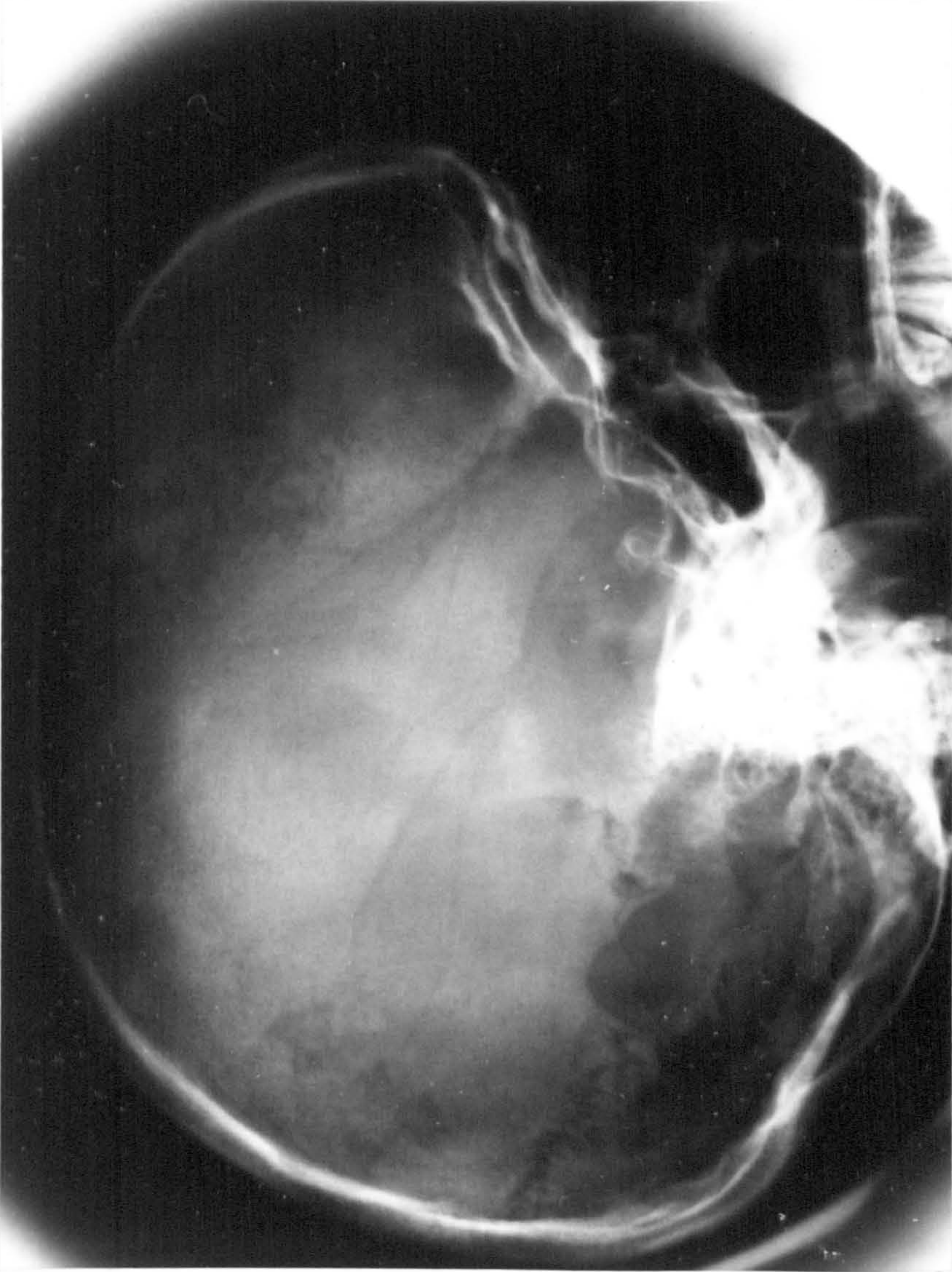


FIGURE 5-3. Bony defect of left lambdoidal suture, similar to that seen in case 119.

abnormality.

(iii) Skull abnormalities - There are two skull defects which are virtually unique to NF-1. These are congenital absence of the sphenoid wing (Holt & Wright 1947; Klatte *et al* 1976; Savino *et al* 1977) and bony defect of the lambdoidal suture (Figure 5-3; Davidson 1966; Klatte *et al* 1976). The former is frequently associated with an orbital plexiform neurofibroma but may occur in isolation, the patients then present with pulsatile enophthalmos.

In the present study no cases of sphenoid wing dysplasia were identified. Case 119 had a lambdoidal suture defect which was picked up on a routine skull X-ray after an accident. However, the author only became aware of this manifestation of NF-1 towards the end of the study and the lambdoidal suture was not routinely palpated on examination.

(iv) Vertebral scalloping and lateral thoracic meningocoele - These manifestations of NF-1 are usually asymptomatic (Heard & Payne 1962; Erkulvrawatr *et al* 1979) and as routine radiology was not undertaken their frequency cannot be estimated. Vertebral scalloping is a descriptive term used when the posterior wall of the vertebral body is markedly concave; it is unclear whether the primary defect is a bone dysplasia leading to erosion of the bone by normal pulsations of CSF pressure transmitted through the dura, or dural ectasia, with an expansion of the dural sac eroding the vertebral canal (Heard & Payne 1962; Caselman & Mandell 1979). The majority of reported cases of lateral thoracic meningocoeles have been in patients with NF-1. They are usually detected as coincidental findings on chest X-ray and it has been postulated that they arise from similar mechanisms to

(A)

(B)



(C)

(D)

FIGURE 5-4. Vertebral scalloping and dural ectasia. Plain lateral radiographs of lumbar spine (A,C) and myelograms (B,D) of case 5. A and B were taken age 20 years, C and D at 44 years, and there has been some progression in the degree of vertebral abnormality.

vertebral scalloping (Erkulvrawatr *et al* 1979). The important differential is from spinal neurofibromas with a "dumbbell" appearance.

The only previous study in which routine radiology was undertaken was that of Riccardi & Eichner (1986); they comment on a frequency of 10% for vertebral scalloping in the lumbar region but do not specifically comment on thoracic meningoceles; it is therefore presumed none were identified.

Case 5 in the present study was previously reported as case 4 in the study of Heard & Payne (1962); the vertebral scalloping in her lumbar spine is shown in Figure 5-4.

E. Malignancy with established disease association - None of the 135 cases in the study area had a malignancy known to be associated with the disease, but this complication had occurred in 3 deceased relatives and one who lived outside the study area (Table 5-2). The frequency of this complication was estimated using the same denominator as for gliomas.

(i) Rhabdomyosarcoma - Two children had died from pelvic rhabdomyosarcomas (case 139 at 5 months, case 140 at 2 years) giving a frequency of 2/138 (1.5%). The site of these tumours is of particular interest, as in the previously reported series (Hope & Mulvihill 1981; Hartley *et al* 1988), in 9/16 children reported the tumours arose in the pelvis.

(ii) Peripheral nerve malignancy - Two relatives (cases 141 and 142) had malignant tumours of peripheral nerves; one had died from a neurofibrosarcoma at 24 years and the other, who lived outside the study area and was not personally examined, had a low-grade malignant

neurilemmoma removed at 33 years, with no recurrence to the time of the study (4 years later). The frequency of peripheral nerve malignancy in this cohort is therefore 2/138 (1.5%), which is similar to that in most previous surveys (Table 1-3) except that of Brasfield & Das Gupta (1972). They reported a frequency of 29% which must be considered a significant over-estimate, resulting from ascertainment bias introduced when cases are identified solely through specialist hospital records.

(iii) Other disease-related malignancies - No cases of neuroblastoma, Wilms tumour or childhood leukaemia were seen in the 69 population study families. The association of childhood leukaemia (with an excess of non-lymphocytic cases) with NF-1 is now accepted, although a definite increase of neuroblastoma and Wilms tumour has not been definitely proven (Hope & Mulvihill 1981; Riccardi & Eichner 1986). The finding of one case who died from Wilms tumour (NF 73, IV-2) in the families ascertained via LINK throws no further light on the matter.

F. Endocrine complications

(i) Endocrine tumours - The association of phaeochromocytoma and NF-1 is long established; 7/72 (9.7%) in one series of patients with phaeochromocytoma had NF-1 (Modlin *et al* 1979). In previous large series of NF-1 sufferers, the frequency of phaeochromocytoma has varied from 0/238 (Riccardi & Eichner 1986) to 3% (Samuelsson 1981). During the course of the present study two papers reported a possible association of duodenal carcinoid tumours with NF-1 and pointed out that these tumours often occurred in association with

phaeochromocytomas (Hough *et al* 1983; Griffiths *et al* 1983). In a more recent review, Griffiths *et al* (1987) presented a much larger series: 27/29 cases of carcinoid tumours in NF-1 were duodenal (at the ampulla vater) and phaeochromocytoma was present in 6/27. With regard to the pathology of the carcinoid tumours, the tumours are glandular, with frequent local infiltration and are frequently confused with adenocarcinomas. The tumours are also rich in somatostatin, although the majority of cases in the series of Griffiths *et al* (1987) presented with bile duct obstruction rather than symptoms of excess somatostatin secretion (diarrhoea, cholelithiasis, mild diabetes and dyspepsia).

In the present study, case 61 had a phaeochromocytoma and at operation an asymptomatic duodenal carcinoid was found. Case 118 was said to have had a duodenal adenocarcinoma removed, following study assessment the histology was reviewed (by Professor E.D. Williams) and found to be a carcinoid tumour. At study assessment case 118 was normotensive and her urinary catecholamines were normal. Both of these cases are included in the report of Griffiths *et al* (1987). In reviewing previous large studies of NF-1 sufferers, one patient is reported with both tumours in the 39 year follow-up of Borberg's cases (Sorenson *et al* 1986). It is likely that the association of duodenal carcinoid with NF-1 is significant and it is important to look for carcinoid tumours in patients presenting with phaeochromocytoma and vice versa.

(ii) Premature/delayed sexual development - Two males in the study (82,85) had had endocrinological investigations for delayed puberty and no underlying abnormality demonstrated, both had subsequently developed normal secondary sexual characteristics. This frequency of

2% for delayed puberty is within the general population range (Tanner and Whitehouse 1976). Although sexual precocity as a complication of optic gliomas encroaching upon the hypothalamus is a well-recorded finding (Holt 1978), whether disturbances of sexual maturation without an underlying cause occur in NF-1 is uncertain. Riccardi & Eichner (1986) formally recorded the onset of puberty in 112 patients and found no variation from the normal population.

G. Cardiovascular complications - The manifestations of cardiovascular problems in NF-1 can be either secondary to external compression by neurofibromas or due to a primary arterial dysplasia which most frequently manifests as either renal artery stenosis (Halpern and Currarino 1965; Salzer & Salzer 1974; Zochodne 1984) or cerebrovascular disease (Taboada *et al* 1979; Levisohn *et al* 1978). In the present series two cases (67, 94) had renal artery stenosis presumed secondary to arterial dysplasia; no cases of cerebrovascular disease related to NF-1 were identified. The occurrence of these two complications is not mentioned in most previous large studies of NF-1; Riccardi & Eichner (1986) report a 1% frequency of renal artery stenosis and although they mention cerebrovascular disease as being an important manifestation of NF-1, they do not state the frequency with which it occurred in their series.

H. Symptomatic neurofibromas (other than peripheral and spinal) - Three individuals had developed symptoms from gastrointestinal neurofibromas. A mother (41) and daughter (42) had both presented with abdominal pain and altered bowel habit (aged 78 and 51 years respectively). In one the tumour was in the appendix and in the

other within the terminal small bowel; in both cases other small lesions were noted at operation either on the surface of the bowel or in the mesentery. Case 19 presented aged 7 years with anorexia and malaise and was found to have a large neurofibroma in the mesentery of the right colon.

The only other case with symptomatic neurofibromas elsewhere was case 5, who had had 5 lesions in her gums for a number of years; these caused occasional pain but no other problems.

I. Congenital glaucoma - A distinctive form of congenital glaucoma is associated with NF-1 (Grant & Walton 1968; Satran *et al* 1980) and is frequently associated with an orbital plexiform neurofibroma. However, cases of congenital glaucoma in isolation are also seen and the primary mechanism is uncertain, although dysplasia of the angle or invasion with neurofibromatous tissue are the most favoured hypotheses (Grant & Walton 1968). Case 71 in this study had unilateral congenital glaucoma described as 'characteristic of NF-1' by her ophthalmologist. In their series, Riccardi & Eichner (1986) report a frequency of 0.5%.

J. Overview of frequency of complications - Direct comparison of the present study with the previous large studies of the disease are difficult either because different forms of neurofibromatosis were not distinguished (Crowe *et al*, 1956; Samuelsson 1981) or ascertainment bias was introduced into the survey (Borberg 1951; Brasfield & Das Gupta 1971; Riccardi & Eichner 1986), or both (Carey *et al* 1979). Although in this study an attempt was made to avoid bias by ascertaining all cases within a geographically defined

population, the frequency of complications may still be an overestimate because of failure to ascertain mildly affected cases in whom a medical opinion had not been sought. Analysing the frequency of complications is one way of correcting for this potential bias; however this group is not truly representative as survey methodology usually selects for cases with complications. In this study, no significant difference was found in the frequency of delayed puberty, epilepsy, intellectual handicap, minor scoliosis or plexiform neurofibromas between affected relatives or index cases, complications which either would not necessarily have led to hospital referral or have been identified through our search of hospital records. The true frequency of NF-1 complications however will only be established as large cohorts of disease sufferers are followed from childhood.

3. Other diseases observed in the study population

These are summarised in Table 5-3. The majority must be presumed to be entirely coincidental findings. However, both juvenile xanthogranuloma (Jenson *et al* 1971; Riccardi & Eichner 1986), cutaneous haemangiomas (Wertelecki *et al* 1982) and hypertrophic obstructive cardiomyopathy (Lin & Garver 1988) have been reported as occurring in excess in patients with NF-1, based on reports of single or small series of cases with both conditions.

4. Disease mortality

The contribution of NF-1 to mortality has been indirectly estimated first by looking at the variation in disease prevalence

Table 5-3 Other diseases observed in the study population

	Study No.	Previous reported association with NF-1
Juvenile xanthogranuloma	104	YES
Cutaneous haemangioma	122	YES
Hypertrophic obstructive cardiomyopathy	108	YES
Carcinoma of breast	52 (σ)	NO
Carcinoma of penis	117	NO
Asthma	29,30,55,127,128	NO
Senile dementia	32,40	NO
Multiple sclerosis	42	NO
Gilbert's disease	105	NO
Congenital heart disease (patent ductus arteriosus)	106	NO

with age (Figure 5-5). Age-specific population figures are not available for the 9th decade and the low prevalence in those aged under 10 years is probably explained by under-ascertainment of sporadically affected children without complications. The decrease in prevalence from the second decade onwards cannot be accounted for solely by under-ascertainment.

Mortality attributable to NF-1 can also be assessed from the cause of death in the 25 deceased relatives (Table 5-4); this was attributable to NF-1 in 6 (22%). A further 6 cases died of malignancies not known to be associated with NF-1. Twelve of the remaining 13 cases died from causes probably unrelated to NF-1; one died from subarachnoid haemorrhage in association with hypertension of unknown aetiology at the age of 48 years.

The pattern of age-specific prevalence (Figure 5-5) and causes of death (Table 5-4) suggest an increased mortality in NF-1 although our study was not designed to assess this statistic. Mortality due to the disease has only been reported by Sorenson *et al* (1986), based on a 39-year follow-up of cases first identified by Borberg (1951). Survival rates to June 1983 of patients who were alive on January 1st 1944 were lower than if their year-, age- and sex-specific death rates had been those of the general population. Mortality was increased among probands, especially females, compared with affected relatives; female relatives had a survival rate just below that of the general population. Borberg's probands were originally identified through hospital in-patient records and the authors concluded that patients requiring hospitalisation have a poor prognosis, whereas incidentally diagnosed relatives may have a considerably better outcome. This study also showed that NF-1

patients with one malignancy (including CNS tumours and phaeochromocytoma) appear to have an increased risk of developing a second malignancy; this occurred in 16/70 (23%) patients whereas a second cancer develops in only 4% of persons with malignancy in the general population (Storm *et al* 1985). As in the case of complication frequencies, a more exact picture of disease related mortality will only be determined by long-term prospective studies.

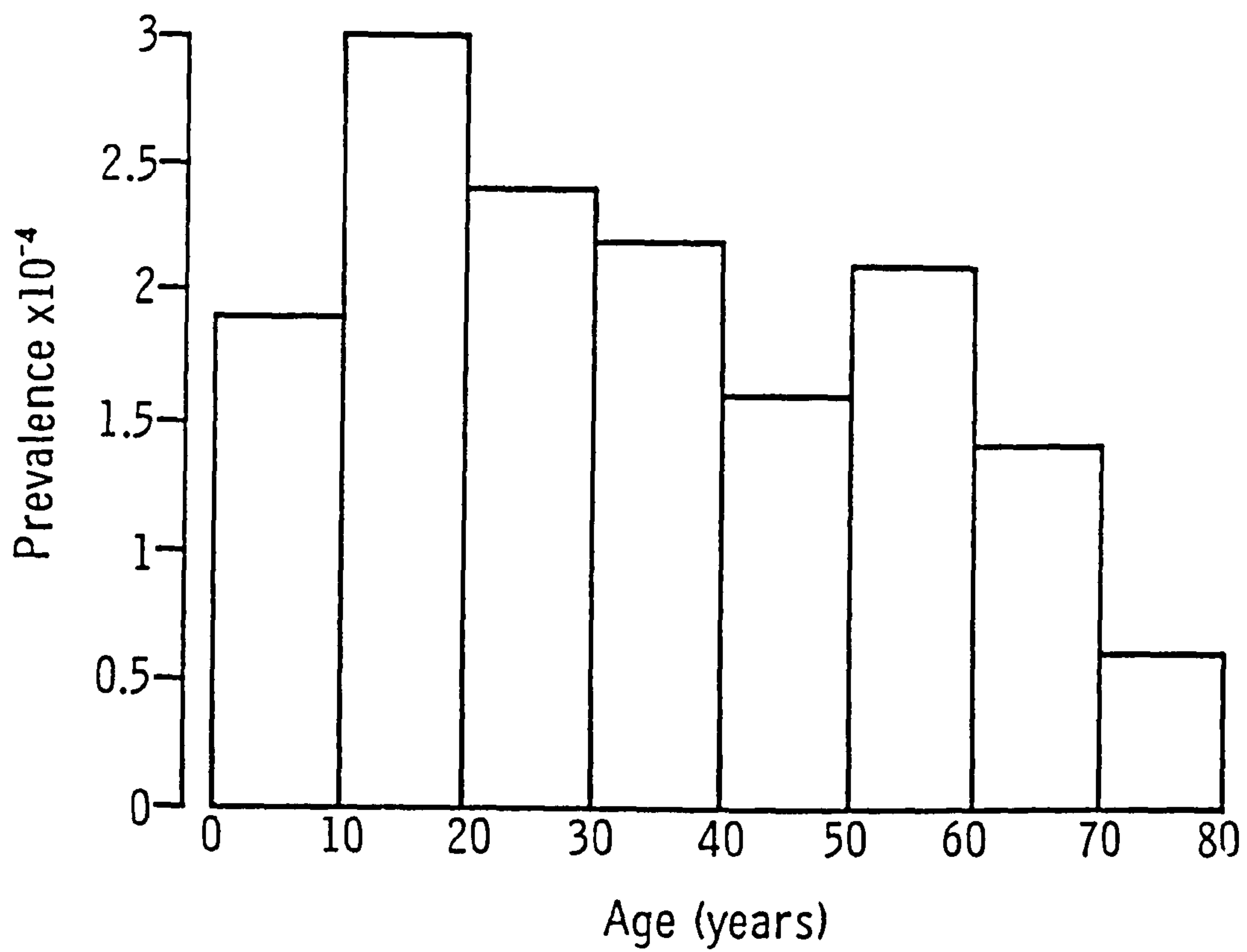


FIGURE 5-5. Difference in the estimated prevalence of NF-1 with age.

Table 5-4 Causes of death in 25 deceased affected relatives

Case No.	Sex	Age (years)	Cause
<u>Related to underlying disease:</u>			
137	F	32	Astrocytoma
139	F	0.5	Rhabdomyosarcoma
140	M	2	Rhabdomyosarcoma
141	F	24	Neurofibrosarcoma
143	F	51	Obstructive hydrocephalus following removal of neurofibroma at C1-2
144	F	54	Acute left ventricular failure and haemorrhage into undiagnosed phaeochromocytoma
<u>Possibly related to underlying disease:</u>			
163	F	48	Subarachnoid haemorrhage, hypertension (aetiology unknown)
<u>Malignancy with no definite disease association:</u>			
145	F	55	Ca cervix
146	F	68	Ca stomach
147	M	60	Ca stomach
148	F	43	Ca breast
149	F	49	Ca lung
150	F	61	Leukaemia (type unknown)

Table 5-4 (continued)

Remaining cases:

151	M	66	Bronchopneumonia, fractured femur
159	M	82	Bronchopneumonia, senile dementia
153	M	57	Ischaemic heart disease
155	F	71	Ischaemic heart disease
154	M	45	Myocarditis
162	M	68	Aortic valve disease
152	M	52	Haematemesis ?cause, mentally subnormal secondary to untreated hydrocephalus
157	M	63	Cerebrovascular disease
158	F	70	Chronic bronchitis
160	F	31	Pulmonary infarction following childbirth
161	M	62	Pulmonary embolus secondary to deep venous thrombosis
156	M	27	Suicide

1. Introduction

The last decade has seen increasing awareness of the problems encountered by NF-1 sufferers; prior to this, although the individual complications were frequently reviewed in the medical literature, no consideration was given to overall care of the patient or whether screening investigations were necessary. As discussed in Chapter 1, a significant factor in the increasing awareness of the medical profession has been the formation of Lay Societies for neurofibromatosis sufferers and their families.

The British neurofibromatosis association, LINK (Let's Increase Neurofibromatosis Knowledge) was formed in 1981; the most frequent complaints of new members (C. Peperell, personal communication) is that they had been given inadequate information about the disease by their doctors and that no one person was prepared to coordinate their health care.

The membership of Lay Societies present a biased group, with a majority of severely affected individuals. The study population, however, presented an unbiased group of NF-1 sufferers in which to assess the care and counselling of such patients. In this chapter the outcome of this analysis is presented, and arising from this, guidelines for the management and counselling of NF-1 sufferers are proposed.

2. Patient care in the South East Wales population

Although a section on patients' attitudes to the disease was included in the study questionnaire (Appendix C), this was often not completed as it had already become obvious that the patient understood so little about NF-1 that the answers would have been uninterpretable. Many of the patients regarded NF-1 as "just a skin complaint", and those with complications often had no idea they were related to NF-1. Those who had sought to inform themselves often had an exaggerated impression of their risk of malignancy or fear of becoming an "Elephant Man". However, the data obtained were sufficient to look at by whom, and at what age the diagnosis of NF-1 had been made, the medical care received by the patients and the number who had received formal genetic counselling.

A. The diagnosis of NF-1 - The results presented in Chapter 4 show that CAL spots are present in NF-1 sufferers by the age of 5 years. If 6 CAL spots are found on routine examination in childhood, NF-1 is by far the most likely cause. The 'awareness' of the medical profession to the diagnosis of NF-1 has been indirectly assessed by looking at by whom and at what age the diagnosis of NF-1 had been made in the 135 individuals in the study population. The outcome is presented in Table 6-1. Twenty of the 135 cases were diagnosed for the first time when assessed for the study. A further 10 individuals, all members of family NF 49, were aware of having the disease through discussion within the family but, regarding it as 'just a skin complaint', had never sought medical advice.

Table 6-1 The 135 affected individuals in the study population
divided according to who had made the diagnosis of NF-1

Diagnosed by	No of cases	No. for whom age at diagnosis known	Mean age at diagnosis in yrs (range)*
Paediatrician	26	23	3.7 (0.3-15)
Dermatologist	21	18	23.0 (0.5-41)
Study**	20	20	22.1 (1-83)
General Practitioner	16	10	37.6 (4-67)
Neurologist	12	8	26.8 (12-46)
Family **	10	-	-
Orthopaedic Surgeon	9	9	12.8 (1-31)
General Physician	7	5	30.8 (18-54)
Plastic Surgeon	7	7	12.4 (1-24)
Ophthalmologist	3	3	4,7,35
Neurosurgeon	2	2	4,33
Clinical Geneticist	1	1	4
General Surgeon	1	0	-

* For numbers <5, age at diagnosis individually listed

** See text for explanation

Table 6-2 The 41 affected individuals aged <18 years in the study
population divided according to who had made the diagnosis

Diagnosed by	No. of cases	Mean age at diagnosis in years (range)
<hr/>		
Paediatrician	20	2.7 (0.3-10)
Study	11	8.3 (1-15)
Family	4	-
Clinical Geneticist	1	4
Plastic Surgeon	1	1
Ophthalmologist	1	4
Orthopaedic Surgeon	1	11
Dermatologist	2	0.5,15

The mean age of diagnosis in the remaining 105 cases was 17.7 years. The majority of cases had not been diagnosed until early adulthood when the appearance of neurofibromas had caused them to seek medical attention. In view of the improved child health surveillance in recent years, the data have been analysed separately for those aged <18 years (n=41) and is presented in Table 6-2. It can be seen that the findings are similar to those for the whole study population. Fifteen out of 41 children had not been diagnosed prior to the study and in 6/27 of the remainder a major disease complication had been the mode of presentation. In a further 4 cases the diagnosis was only made during a hospital consultation for unrelated medical problems. On a purely subjective level, many parents said that they had pointed out the CAL spots to various doctors only to be told they were 'just birth marks'.

B. Patient care and genetic counselling - 94/135 (70%) cases in the study population had had at least one hospital consultation for NF-1 prior to their assessment for the study. Yet only 30/135 were being regularly followed in a hospital clinic, and in half of these it was to monitor a specific disease complication (Table 6-3). None of the remaining 105 individuals were under regular review with their general practitioner for NF-1. The medical histories of many of the cases (Appendix A) demonstrate that regular follow-up with more information about the disease would have avoided delay in diagnosis of complications and distress caused by uncertainty. The cases with hypertension secondary to renal artery stenosis (67, 94) or phaeochromocytoma (61) illustrate clearly the value of regular follow-up. In all three of these cases, the raised blood pressure

Table 6-3 Reason for hospital follow-up in 30/135
individuals in study area

Reason	Number
<hr/>	
General overview of disease	15
- Adults	5
- Children (<18 years)	10
Because of disease complication	15
- Neurological complications	5
- Facial plexiform neurofibromas	3
- Ophthalmological complications*	3
- Orthopaedic complications	2
- Endocrine complications	1
- Follow-up after GI surgery	1

* Including optic gliomas as both individuals with this complication followed only by an ophthalmologist.

was a coincidental finding on examination when they had presented with another problem.

Only 9 individuals from 7 families had received formal genetic counselling, and in 4 cases this was after they had completed their families. Although others had been informed of the mode of inheritance, the variation of disease severity and risk of complications had not been explained.

3. Guidelines for the management and counselling of NF-1 sufferers

The data presented in Section 6-2 shows that there is a need for improved patient care in NF-1. Although many complications are individually rare, their combined burden is significant and it is likely that sufferers are not being diagnosed sufficiently early, nor receiving appropriate follow-up and counselling. Based on the findings of the study, guidelines for genetic counselling and patient management in NF-1 are now presented.

A. Genetic counselling - The information needed in genetic counselling of NF-1 sufferers can be divided into three main areas:

(i) Recurrence risks for parents of an isolated case - The reports of cases with limited expression of the NF-1 gene having affected children (reviewed by Riccardi & Lewis 1988) means that detailed cutaneous and slit lamp examination of the parents of an apparently isolated case is essential before counselling is given. In the absence of any abnormalities, there is no significant increased risk of a further affected child. The author is aware of only 2 cases of recurrence with apparently normal parents (Riccardi & Lewis 1988).

(ii) Assessment of at-risk children - The data presented in Chapter 4 show that, in assessing children of an affected patient, those who have inherited the gene can be distinguished from their normal siblings on the basis of whether or not CAL spots are present by the age of 5 years.

(iii) Risk figures for prospective parents - The 50% risk of autosomal dominant inheritance is straightforward; what is more difficult is explaining the risk of complications. Informing sufferers and their families about the varied complications of NF-1 is a difficult counselling task, and there is a fine balance between providing adequate information and causing unnecessary alarm. Rather than presenting patients with a long list of complications, it is probably more useful to group the complications according to the effect they will have on an individual's life as shown in Table 6-4. The risks to offspring of an affected parent are therefore: intellectual handicap, 16.5% (moderate/severe retardation, 1.5%, minimal retardation/learning difficulties 15%); complications developing in childhood and causing lifelong morbidity, 4.5%; 'treatable' complications which can develop at any age, 8%; and malignant or CNS tumours, 2-3%. The risk for a given complication group has been halved and rounded to the nearest 0.5%.

B. Patient management - Based on the experience of this study, it is recommended that patients with NF-1 have regular clinical assessments, although none of the complications occur often enough to warrant biochemical or radiological screening. As many of the

Table 6-4 Frequency of complications for counselling purposes

Complication Group	Frequency
<hr/>	
1. Intellectual handicap:	
moderate-severe retardation	3.2
minimal retardation or learning difficulties	29.8
	33.0
2. Developing in childhood and causing lifelong morbidity:	
severe plexiform neurofibromas of head & neck	1.2
scoliosis, requiring surgery	5.2
severe pseudoarthrosis	2.1
	8.5
3. 'Treatable' complications which can develop at any age:	
aqueduct stenosis	2.1
epilepsy	4.2
spinal neurofibromas	2.1
visceral neurofibromas	2.1
endocrine tumours	3.1
renal artery stenosis	2.1
	15.7
4. CNS and malignant tumours:	
optic gliomas	0.7
other CNS tumours	0.7-1.5
rhabdomyosarcoma	1.5
peripheral nerve malignancy	1.5
	4.4-5.2

complications develop early in life, children with the disease should have biannual review, particular attention being paid to intellectual development so that the child can be channelled into appropriate schooling. In adults, unless a particular complication indicates more frequent review, annual clinical examination only is recommended.

In Figure 6-1 the age ranges for presentation of the majority of NF-1 complications are given. It is intended that this can be used when seeing patients to assess what complications an individual of a given age is still at risk of developing. This can be used both for reassurance, for example a young adult with no problems to date can be reassured they have no risk of certain complications, and in monitoring for complications which may yet occur. The age range during which a given feature may present has been derived as follows, from review of previously published series: aqueduct stenosis (Horwich *et al* 1983), endocrine tumours (Modlin *et al* 1979; Griffiths *et al* 1987), CNS tumours (Illgren *et al* 1985, Lewis *et al* 1984), pseudoarthrosis (Morrissy *et al* 1981), rhabdomyosarcoma (Hartley *et al* 1988; Hope & Mulvihill 1981) and scoliosis (Chaglassian *et al* 1976; Winter *et al* 1979); from the age of presentation in this study and that of Riccardi & Eichner (1986): plexiform neurofibromas; for intellectual handicap it was assumed that severe development delay would have been identified by the age of 5 years and milder problems by the end of primary school education; for the remaining complications given as having a 'lifelong' risk of presentation, examples of each occurring from childhood throughout life were found either in this series or in the literature. Insufficient data are available to provide a more accurate age range.

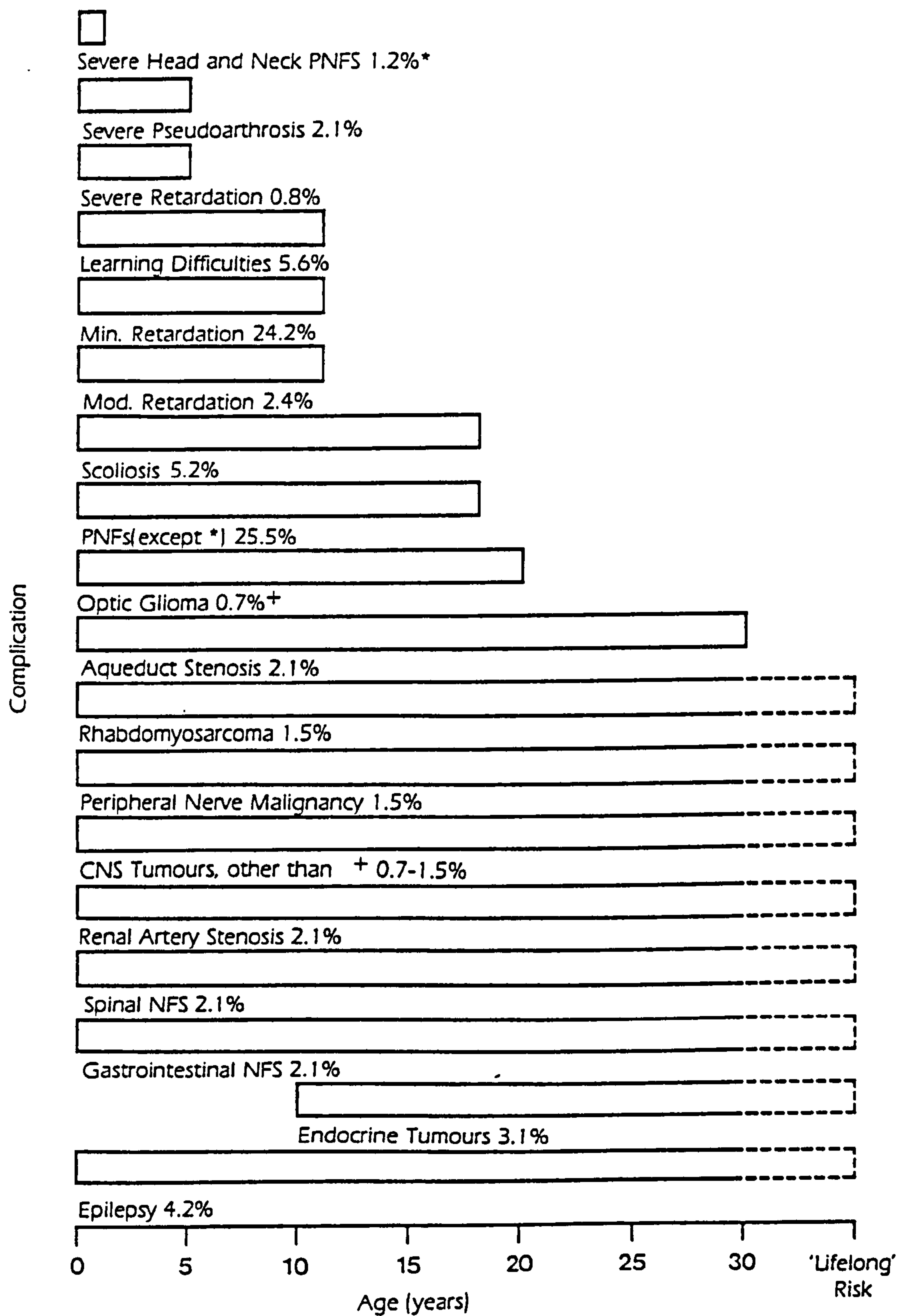


FIGURE 6-1. Age range of presentation and frequency of major NF-1 complications. The following abbreviations have been used: PNFS for plexiform neurofibromas and NFS for neurofibromas.

Which specialty should take on the responsibility of follow-up of NF-1 sufferers is open to debate. The paediatric clinic is clearly appropriate for children, but in adults the general practitioner or any one of a number of specialists (e.g. clinical geneticist, neurologist or dermatologist) are equally appropriate. There may be a case for a few centres in the UK establishing specialist neurofibromatosis clinics, so that particular expertise can be developed in treating some of the more difficult or unusual complications, and in the assessment of atypical cases. In Figure 6-2, an outline of how such clinics would interact with 'local' clinics is illustrated. Although one person would have overall charge for the specialist clinic, they would need to identify colleagues in a number of specialties to assist in the management of different complications. The clinic would also be an ideal setting for evaluation of new diagnostic techniques and treatments and for the coordination of long term follow-up studies of NF-1 sufferers. Through such studies, clinical features which predispose to the development of certain complications (e.g. malignancy) may emerge, thus identifying a sub-population of NF-1 sufferers who require closer follow-up.

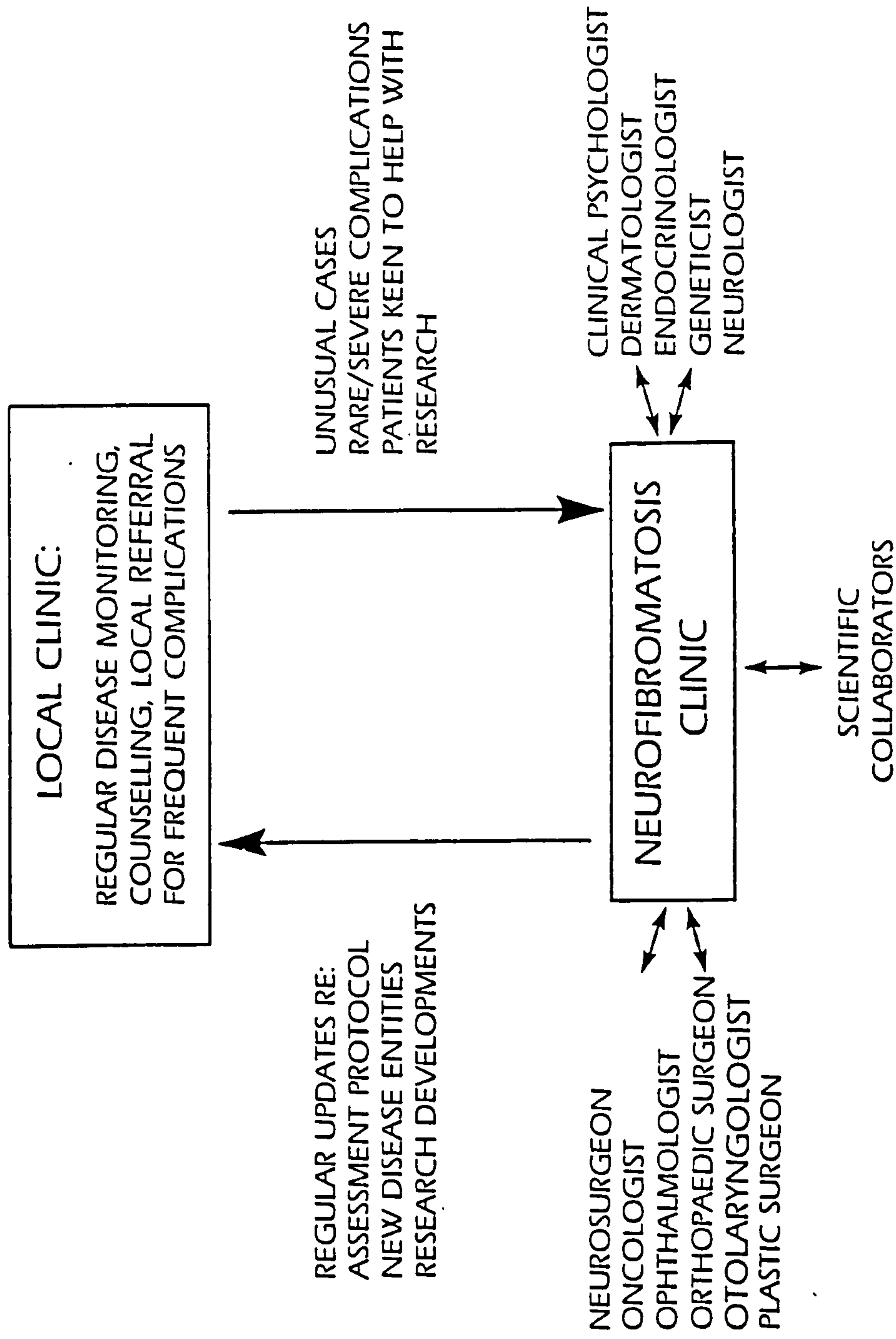


FIGURE 6-2. Proposed plan for organisation and interaction with local centres of specialist neurofibromatosis clinics.

PART 2

GENETIC LINKAGE STUDIES OF VON RECKLINGHAUSEN NEUROFIBROMATOSIS

CHAPTER 7 Introduction to human gene mapping and review of
literature pertinent to the possible localisation
of the NF-1 gene

1. Introduction

Since 1970 a series of discoveries in the field of molecular biology have led to a complete reorientation and renewed interest in research into human genetic disease. Through these discoveries we are now able to look at genetic disease not just on a cellular and biochemical level but on a molecular level. For a disease like NF-1 it has made it possible to consider localising and eventually isolating the gene without knowing the gene product. This chapter briefly outlines the DNA techniques used in gene mapping, then reviews the principal methods used to map genes and finally the possible 'clues' to the localisation of the NF-1 gene at the outset of this work are reviewed.

2. Recombinant DNA technology and the study of human genetic disease

The fundamental principle on which most methods of DNA analysis of human genetic disease are based is the ability of small amounts of pure labelled DNA to hybridise to a much larger amount of genomic DNA. The labelled DNA is used as a 'probe' to study its complementary sequence in the genomic DNA of the individual under study. The DNA probe may be all or part of a gene known to cause a particular disease and used to study the rearrangements of that gene in affected patients. Alternatively random unique sequence DNA

probes which identify variation within the normal population can be used in linkage studies. The actual methods by which DNA probes are used in hybridisation experiments are described in detail in Chapter 8. The developments which made this approach possible are briefly reviewed here. For more detailed reviews, see Watson *et al* 1983; Old & Primrose 1985; Weatherall 1985.

(A) Restriction endonucleases - These are a group of bacterial enzymes which cut double stranded DNA where they recognise particular base sequences, usually 4-6 in length. In bacteria their action is to break down foreign DNA. The first of these was described in 1970 (Smith and Willcox; Kelly and Smith) and since that time over 400 have been isolated. Their importance is that they have given molecular biologists a way of cleaving genomic DNA into small fragments of known size suitable for cloning and hybridisation experiments. Furthermore, the finding of variation between individuals in the presence/absence of sites where these enzymes cut the DNA has given rise to a new class of genetic markers, restriction fragment length polymorphisms, which are described in section D.

(B) Cloning of DNA fragments - The development of these techniques was fundamental to recombinant DNA technology. The principle is to insert foreign DNA into a suitable vector molecule which serves to propagate that DNA segment in a bacterium such as *E. coli*. The vectors used are plasmids, phages and cosmids. The choice of vector depends upon the size of the DNA to be cloned. A detailed account of DNA cloning is given by Old and Primrose (1985).

Cloning vectors usually have two or more single sites for different restriction endonucleases. Generation of a recombinant molecule involves cleaving donor and vector DNA using the same restriction enzyme; they are then mixed in the presence of a DNA ligase which promotes the rejoining of the cut strands of DNA. Some vectors reanneal to reconstruct their original form but others form recombinants by incorporating the foreign DNA into their structure. The vectors containing recombinant DNA molecules are then introduced into a bacterial host, usually *E. coli*; the bacteria are then cultured, resulting in large amounts of the recombinant vectors being produced. The recombinant DNA can then be retrieved from the bacteria, the final product being large amounts of the original 'foreign' DNA for use in hybridisation experiments (Maniatis *et al* 1982).

(C) Gene libraries - Using the techniques of DNA cloning it has become possible to construct gene "libraries" which are the usual source of single copy DNA sequences for use as probes. Gene libraries may be of 3 main types:

(i) Complementary DNA (cDNA) - cDNA libraries can be made from particular tissue or cell types, cDNA copies of the cells' RNA being cloned and the clones therefore representing all the active genes of that particular specimen (Williams 1981).

(ii) Genomic libraries - A genomic library consists of total cellular DNA digested with a restriction endonuclease and recombinants prepared between the fragments thus created and plasmid or phage

treated with the same enzyme. A total human DNA library consists, therefore, of hundreds of thousands of clones, each representing a different piece of the genome (Lawn *et al* 1978).

(iii) Chromosome specific libraries - These are of particular use when the chromosomal localisation of the disease under study is known. The chromosome of interest can be separated by flow-sorting and used to prepare recombinants or somatic cell hybrids, containing all or part of the human chromosome of interest, can be used. By this method the number of clones to be screened is greatly reduced.

Gene libraries thus created can be used in a variety of ways. If the nucleic acid sequence of a given protein is known, then a synthetic DNA probe can be constructed from the appropriate nucleotides and used to screen DNA libraries for clones containing all or part of the actual gene encoding the protein of interest. Alternatively, libraries can be used as sources of random unique sequence DNA, and these in turn used to detect restriction fragment length polymorphisms for use in genetic linkage studies, as explained in the subsequent section. The earliest example of the success of this approach is the X-library of Davies *et al* (1981) which provided the first DNA probe which identified a random genomic DNA polymorphism linked to a disease locus (Duchenne muscular dystrophy, Murray *et al* 1982).

(D) Application of DNA technology to localisation and isolation of genes

The genetic diseases to which DNA technology was first applied successfully were those where the gene product is known, the haemoglobin disorders being a classical example (Weatherall, 1985).

As the RNA in human reticulocytes is almost entirely globin messenger RNA (mRNA), it was possible to make purified mRNA for single globin chains and thus make cDNA probes. With these it was possible to isolate the globin genes from libraries of human DNA, characterise their fine structure and then compare normal structure with that of DNA from patients with haemoglobin disorders.

These studies have shown that the haemoglobin disorders and structural haemoglobin variants have arisen from a number of DNA arrangements of varying complexity. From the findings of these and related studies, it seems likely that the majority of human genetic diseases will turn out to have resulted from not just one but a variety of DNA rearrangements. The importance of these discoveries clinically has been that families with haemoglobin disorders can be offered DNA diagnosis with a chorionic villous biopsy at eight to twelve weeks of pregnancy, rather than foetal blood sampling in the second trimester.

One of the main ways in which normal genes are compared with abnormal genes is the technique of 'restriction mapping'. The cloned gene is labelled with radioactive phosphorus and used as a DNA 'probe'. Its hybridisation with fractionated human DNA which has been digested with a series of restriction endonucleases is studied and a restriction 'map' of the gene built up. This in turn can be compared with the findings when the probe is used with the DNA from patients with a disorder of that particular gene. If the rearrangement underlying the abnormal gene causes the loss of or creation of a restriction site, the DNA probe will detect this by 'lighting up' segments of different length from normal. In more extreme cases the disease may have been caused by the deletion of the

whole of a region of DNA and this will be detected by the DNA probe failing to hybridise (and therefore 'light up') with a complementary sequence.

It is however relatively uncommon for the actual gene rearrangement itself to affect a restriction enzyme site. Fortunately, as genes were cloned and their restriction maps studied, it became apparent that some restriction sites were polymorphic with no phenotypic effect due to presumed 'neutral' mutation, i.e. they showed variation within the normal population. Therefore, a DNA probe for a given site is able to detect variation therein, as on hybridisation it will pair with differing lengths of DNA i.e. there is polymorphism of restriction fragment length (RFLP). The first human RFLP to be discovered was a HpaI site about 5 kbs from the 3' end of the beta-globin gene (Kan and Dozy, 1978). Further studies of RFLPs showed that they were inherited in a Mendelian co-dominant manner and it was anticipated that they would be fairly evenly distributed throughout the genome and that more variation could be expected in regions between structural genes (Jeffreys 1979).

In 1980, Botstein and his colleagues pointed out the potential usefulness of RFLPs as genetic markers for chromosome mapping. They emphasised that the RFLP need not be within a gene, but could occur in any random unique sequence DNA for which a complementary DNA probe was available. They estimated that if approximately 200 probes that identified RFLPs were available and evenly spread throughout the genome, then all genetic diseases could be mapped. The first RFLP identified by a random unique sequence probe was described by Wyman and White in 1980. The "detection" of an RFLP by a DNA probe is shown in Figure 7-1.

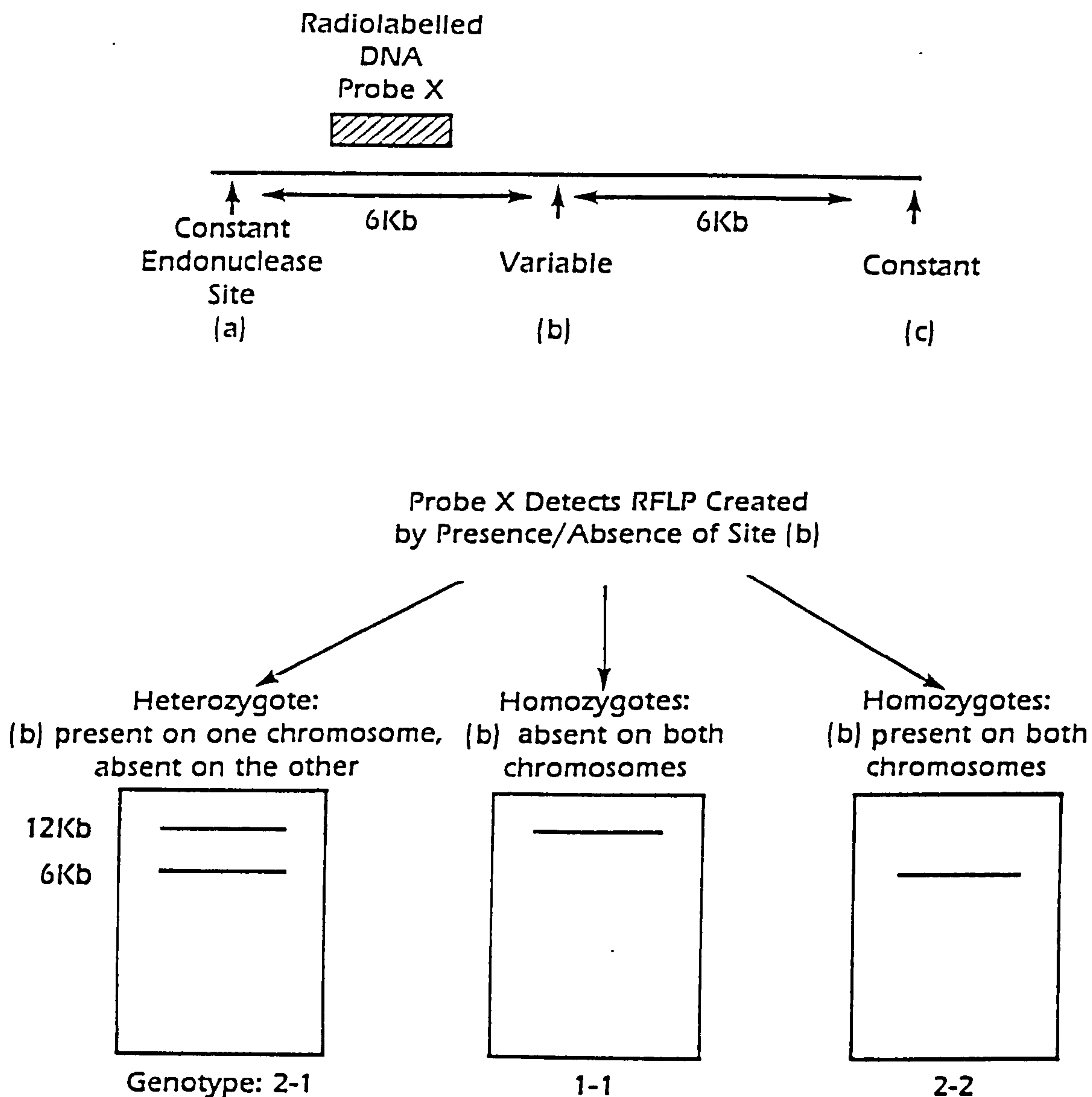


FIGURE 7-1. Diagrammatic representation of detection of a RFLP by a DNA probe and corresponding genotypes on an autoradiograph.

DNA probes can be used in linkage studies in two ways:

- (i) probes for 'candidate' genes (e.g. Nerve Growth Factor for NF-1) can be used to identify RFLPs, the segregation of which can be studied in families, and if cross-overs are found, the gene is excluded as the cause of the disease;
- (ii) random probes which identify RFLPs can be used as genetic markers to look for linkage.

Once the gene has been mapped to a given chromosome using these methods, then recombinant DNA technology provides the means by which, in conjunction with somatic cell hybrids and cytogenetic techniques, 'fine' mapping and eventual isolation of the gene itself are possible (Orkin 1986).

3. Methods of human gene mapping

(A) Family linkage studies -

(i) Historical background - Two genes are said to be linked when they are located on the same chromosome within measurable distance of each other. Genes on the same chromosome that do not show linkage are said to be syntenic (Renwick 1969). In family linkage studies the segregation of "marker" genes, the chromosomal localisation of which is known, is followed through pedigrees in which the disease under study is segregating: if the two loci are closely linked then one allele of the marker gene will nearly always segregate with the disease, thus localising the disease to the same chromosome.

The term linkage was first used by Morgan in 1910 following on from the work of Bateson and his colleagues (1908). In 1865 Mendel

had concluded that the segregation of two different pairs of alleles is independent but soon after the rediscovery of his work in the first decade of this century, Bateson *et al* (1908) found an exception to this rule in the sweet pea. They observed certain combinations of alleles more frequently than expected. The alleles of the parental combination seemed either to attract one another or to repel one another. Bateson *et al* coined the terms "coupling" for the former phase and "repulsion" for the latter phase. Morgan (1910) recognised that coupling and repulsion represent two aspects of the same phenomenon, location of two genes on the same or on homologous chromosomes. He coined the term "linkage". Morgan explained the finding by exchange of chromosome pieces between homologous chromosomes during meiosis (recombination) and recognised that the frequency of crossing over depends upon the distance between the two loci.

In the first two decades of this century, using recombination as an analytical tool, Morgan and his co-workers were able to draw up detailed genetic maps of the four chromosomes of *Drosophila*. In man, however, directed breeding is not possible and therefore statistical methods had to be developed to study human pedigree data. The technique of lod scores, most extensively used today, is described in the second part of this section. Another problem in man is that the occurrence of two genetic traits in the same family is a rare event and so for many years the application of linkage techniques to human gene mapping was very limited. The demonstration of genetic linkage by human pedigree studies was first reported on the X-chromosome by Bell and Haldane (1937) who demonstrated linkage between haemophilia and colour blindness on the X-chromosome.

With the identification of the blood groups and polymorphisms of proteins and enzymes, it was possible to apply their variation within the normal population and to use them as "marker" genes. A useful marker gene shows frequent polymorphism within the general population. Linkage between the markers themselves can be searched for by studying their segregation in human pedigrees, or the segregation of marker genes studied in families with a given Mendelian disorder to try to identify a linked marker. The first autosomal linkage in man was reported by Mohr (1951) between the Lutheran and Secretor loci.

Prior to the advent of recombinant DNA technology the limited number of polymorphic genetic markers meant that there was no systematic method for mapping human genetic disease. Many linkage studies were performed on the more serious autosomal diseases using classical markers but few were fruitful. Since 1980, however, there has been a renewed interest in family linkage studies following the discovery of RFLPs. The clinical usefulness of the approach proposed by Botstein *et al* (1980) was dramatically demonstrated by the mapping of Huntington's Chorea in 1983 (Gusella *et al*) and adult polycystic kidney disease in 1985 (Reeders *et al*). Not only does the localisation of the gene by linkage mean that it should be possible to 'move in' onto the actual gene of interest from the linked RFLP but also, where appropriate, closely linked RFLPs can be used in genetic counselling (Meredith *et al* 1986).

(ii) Methodology of linkage analysis: The aim of any linkage study is to establish whether or not the loci under observations lie within measurable distance of each other. The distance between genes is

measured in centiMorgans (cM), where one cM represents 1% recombination between two loci and in physical terms is thought to be equivalent to one million base pairs. Thus if 5% of the progeny from matings informative at two loci are of the recombinant type, these loci are said to have a recombination fraction (θ) of 0.05. Over short distances the recombination fraction is equivalent to the distance in cM between two genes. This relationship does not hold for larger distances because of the probability of double cross-overs. The maximum recombination fraction between two genes is therefore 0.5, equivalent to random assortment.

Early human linkage analysis was done by the methods of Bernstein (1931) or Penrose (1935) who compared the traits of interest in sib pairs. Nowadays, however, both these methods have been replaced by the method of "lod scores" as developed by Haldane and Smith (1947b) and Morton (1955, 1956, 1957 and 1962). Using this method the probability that the observed family data conform to the behaviour of two loci under full recombination is first calculated, then the probability of the data based on a range of θ from 0.00 to 0.50. The ratio (Pr) of these two probabilities is the likelihood ratio and expresses the odds for and against linkage.

$$\text{Pr} = \frac{P(\text{family, given } \theta = 0-0.5)}{P(\text{family, given } \theta = 0.5)}$$

For convenience, Pr is usually expressed as its logarithm, the \log_{10} of the relative probability is called the "log of the odds" or the lod score.

In a family linkage study the lod score is calculated for each family and these are then added. The maximum likelihood estimate of

θ may be obtained by plotting the sum of the lod scores against the various values of θ and is the value corresponding to the peak of the curve. Because of the known sex difference in frequency of recombination, it being increased in females, the lod scores are also calculated for male and female meioses separately. Autosomal linkage can be considered highly probable when in a collection of families the sum of the lod scores at any value of θ reaches +3 (i.e. odds 1000:1 in favour of linkage); if it reaches -2 (i.e. odds 100:1 against linkage) then linkage is considerably unlikely.

The calculation of lod scores for the non-mathematician was much simplified first by the introduction of tables of lod scores for given pedigree situations (Maynard Smith *et al* 1961) and more recently the development of computer programs. The programs most widely used today are LIPED developed by Ott (1974) and LINKAGE (Lathrop *et al* 1985). Both these programs make maximal use of human pedigree information which was difficult when using extensive pedigrees and the lod score tables.

(B) Somatic cell hybrids The techniques involved in the creation of somatic cell hybrids are based on the observation that somatic cells of the same or of two different species will fuse under certain conditions (see Francke 1983 for more detailed discussion). For the purpose of human gene mapping rodent-human cell lines are generally used. This is because when an established rodent cell line is fused to human fibroblasts or lymphocytes, the human chromosomes will be preferentially eliminated. The initial chromosome loss from fused cells during mitosis is marked but after a short period of time the hybrid cells become stable and retain one or more human chromosomes.

Over longer periods, additional human material may occasionally be lost but regular characterisation of the cell line will monitor this.

When creating hybrid cells, it is also possible to select for the retention of certain human chromosomes. The means used include culture media in which a certain cell type cannot grow or grows preferentially, or selective killing of one cell type with toxins, antibodies or viruses to which other cells are resistant. There are now selection systems for about half the human chromosomes (Francke 1983).

Fortunately, it is not necessary to have 23 hybrids each with one human chromosome for mapping, but a smaller panel of hybrids each with a unique combination of several human chromosomes can be used. Thus a characteristic distribution pattern for each chromosome can be created. Further refinements of these techniques have led to the use of panels of hybrids created from human lines with chromosomal rearrangements, usually translocations. This enables regional mapping of a gene on a given chromosome (see Brook *et al* 1984, for chromosome 19 as an example). Initially only genes expressed in culture could be mapped using hybrids but this problem has been overcome with the developments in DNA technology. The initial assignments using hybridisation panels were of enzymes which could be studied electrophoretically and surface antigens identified immunologically. Using DNA techniques, however, the DNA of the different hybrids of a panel can be fractionated using the appropriate restriction endonuclease, separated on an electrophoresis gel and then hybridised with the DNA probe of interest. This might be a gene or a random piece of DNA which identifies an RFLP and therefore its chromosomal localisation is important. Using a hybrid

panel the localisation of the 'probe' can be deduced.

(C) Cytogenetics and gene mapping - Although cytogenetic techniques play an integral part in mapping using somatic cell hybrids, karyotype analysis per se can result in or play a part in the mapping of a gene in several other ways:-

(i) Marker chromosomes - Typical markers consist of heterochromatic variants, fragile regions or structurally abnormal chromosomes. The first autosomal assignment in man was that of the Duffy locus which was found to segregate with a large variant of the centric heterochromatin in chromosome 1 (Donohue *et al* 1968). The most important example of fragile site association is that on the long arm of the X-chromosome which segregates with the most common form of X-linked mental retardation (Howard-Peebles and Stoddard 1979). With regard to structural variants, an important assignment was of the HLA locus to chromosome 6 by means of a pericentric inversion segregating in a family (Lamm *et al* 1974).

(ii) Gene dosage effects in cases with chromosome deletion/

duplication - Cases with isolated chromosomal deletions can be compared with their parents for variation in polymorphic markers. The first example of this was the mapping of red cell acid phosphatase to chromosome 2. The patient had lost a maternal allele for this enzyme and also had a level of 45% less than expected (Ferguson-Smith *et al* 1973). Assay of enzyme levels is thus the second way these cases can be studied and in cases with chromosome duplication the enzyme levels will be raised.

(iii) Chromosomal deletions/translocations giving clues to

localisation - Perhaps the best example of this has come from the study of the karyotype of females who have a clinical picture indistinguishable from X-linked recessive Duchenne Muscular Dystrophy. Several of these cases have been reported and in each an X-autosomal translocation is present, with the X-chromosome breakpoint in band Xp21 (see Jacobs *et al* 1981 for case report and review). In these cases the normal X-chromosome is inactivated which means that recessive genes on the translocated X are expressed. However if this were the only explanation, the breakpoint should be random rather than always in Xp21 and it was therefore suggested that the DMD locus was at Xp21. DNA techniques have since shown this localisation to be correct (Murray *et al* 1982; Monaco *et al* 1985; Ray *et al* 1985).

(iv) In situ hybridisation - This technique has developed with the advancement of DNA techniques. In this method, metaphase spreads are banded and photographed and then the radiolabelled DNA clone of interest is annealed to the spread, which is then rephotographed. The chromosomes are then re-examined to locate the grains of radioactivity. The first human single genes were localised with this technique in 1981. These were the alpha-globin genes assigned to chromosome 16 (Gerhard *et al* 1981) and beta-globin genes to the short arm of chromosome 11 (Malcolm *et al* 1981).

4. Review of previous reports pertaining to the localisation of NF-1 gene

At the outset of this work in October 1983, there were no clues to the possible gene localisation from patients with chromosome abnormalities and there were only two published reports of linkage analysis. The first of these was the study of Lepage *et al* (1981) which excluded close linkage with the HLA locus. The second was the linkage study of 28 classical markers undertaken by Spence and her colleagues (1983). For 16 of the studied markers the lod scores provided statistically significant evidence against linkage for at least some values of θ . Of the other 12 markers, none gave a lod score >1 which might have encouraged 'a further look' at the particular locus. The only interesting finding was that the GC locus gave a lod score of +2.2 in the first five informative families tested, but the sixth family gave a negative score; the combined lod score was 0.89. These results were tested for linkage heterogeneity, and the results were not statistically significant.

Despite the paucity of published linkage studies of NF-1, there had been two families reported where NF-1 and myotonic dystrophy (DM) appeared to segregate together, suggesting that the two diseases may be closely linked. The first of these families is shown in Figure 7-1 and was reported by Ichikawa and his colleagues (1981); it can be seen that there are 7 individuals in generations II and III with both NF-1 and DM. In generation I the origin of NF-1 is clear (i.e. I-1) but that of DM uncertain. If one assumes that I-1 had both NF-1 and DM, then the lod score from his counted descendants in generations II and III is 2.4. This would be highly suggestive of linkage between

NF-1 and DM. If however, I-2 had DM then the segregation in this family is strongly against linkage. III-9, age 19 yrs, and all members of generation IV were excluded from analysis because of the delayed age of onset of DM. Studies of the segregation of secretor (Se) (Ichikawa *et al* 1981) and the third component of complement (C3) (Pericack-Vance *et al* 1984) polymorphisms in this family supported the clinical observation that the DM in this family was the same locus as in other families.

The second family was presented in poster form at the 7th International Workshop on human gene mapping by Rivas and Di Liberti (1984). In this family there were seven individuals with both disorders in 4 generations, all doubly-affected offspring having a doubly-affected parent. One adult, a 23 year-old female with NF-1 only, appeared to be a possible recombinant. If one assumes she will not develop DM, then the lod score is 0.75 at a recombination frequency of 0.20, BUT if she does develop DM then the lod score would be 3.01 with no recombination.

In neither of these families were any clinical 'peculiarities' reported that might have made one speculate about a new disease in which the features of NF-1 and DM were combined. Therefore, if the clinical assumptions in these families were correct, they provided strong evidence of close linkage between NF-1 and DM which merited further investigation. A study of the segregation of chromosome 19 markers linked to DM in NF-1 families was therefore undertaken.

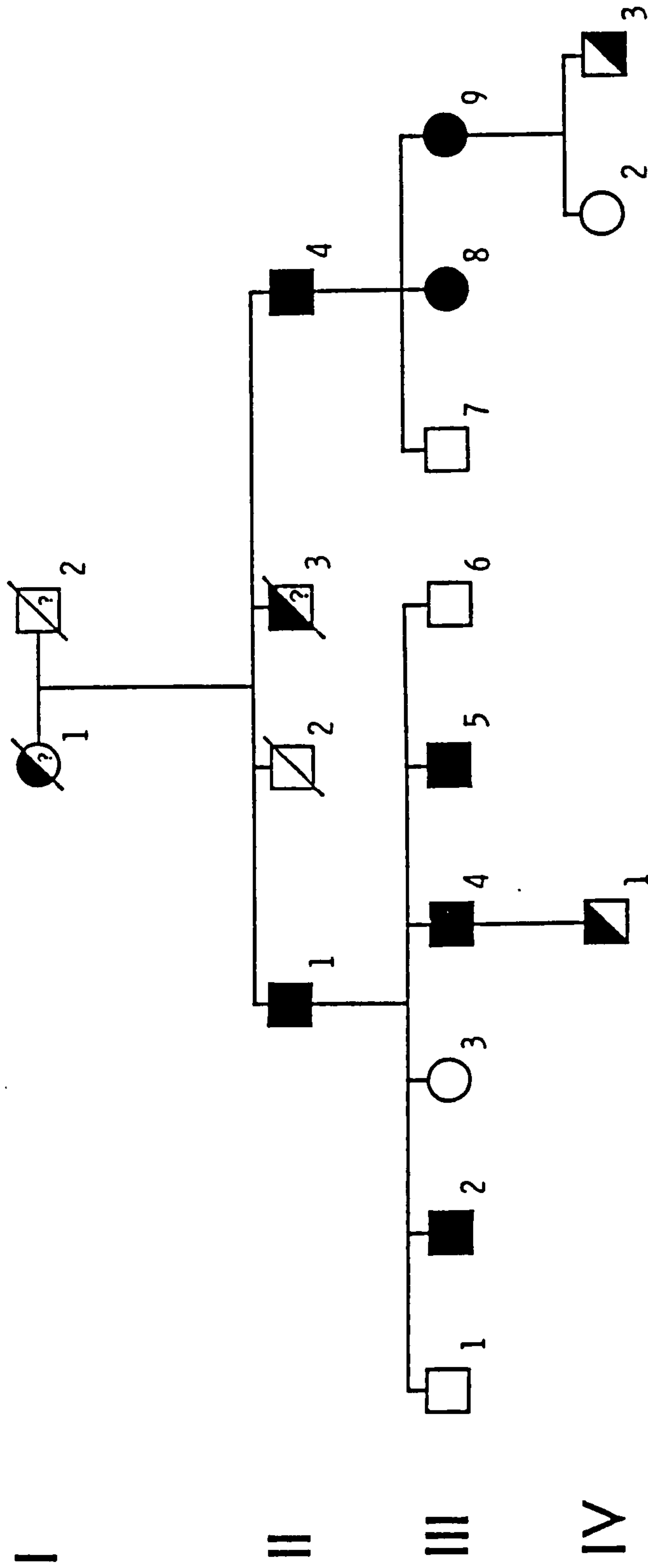
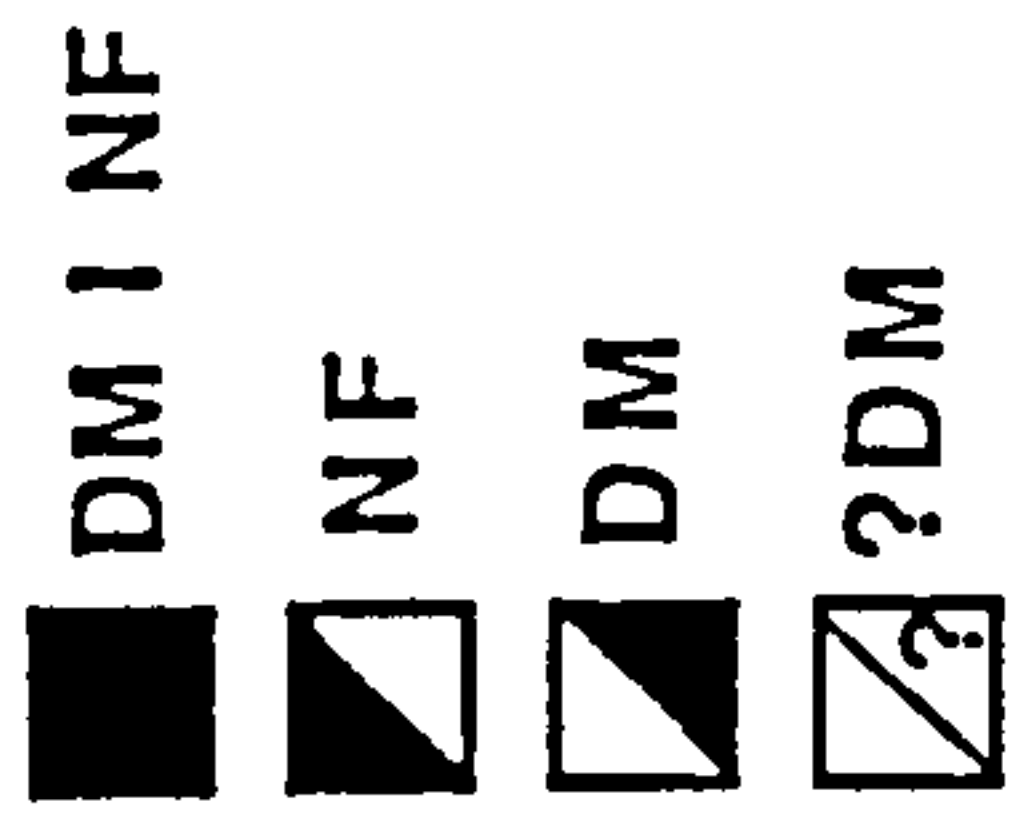


FIGURE 7-2. Apparent co-segregation of NF-1 and DM in the pedigree reported by Ichikawa et al (1983,. adapted from published pedigree with permission).

CHAPTER 8 Genetic linkage studies of NF-1 and chromosome 19

markers linked to DM

1. Introduction

DM was the first serious autosomal dominant disease for which a linked marker was identified. In 1954 Mohr reported a suggestion of linkage between DM, the Lutheran blood group (Lu) and the secretion of ABH blood group substances (Se). This linkage was confirmed by the later studies of Renwick *et al* (1971) and Harper *et al* (1972). Further linkage studies added peptidase D (PEPD, Cook *et al* 1972) and C3 (Eiberg *et al* 1982). The whole linkage group was assigned to chromosome 19 with the assignment of C3 to that chromosome by Whitehead *et al* in 1982. In that year apolipoprotein E (APOE) was also shown to be linked to C3 (Olaisen *et al* 1982). The linkage relationships of APOE to DM however were only studied after completion of this study (Laberge *et al* 1985).

At the outset of the study, therefore, the polymorphic markers known to be within a measurable distance of DM were Lu, Se, PEPD and C3, of which PEPD was the closest (Davies *et al* 1983; O'Brien *et al* 1983), but which is rarely polymorphic. Fortunately, during the period of family identification and collection of specimens, the apolipoprotein CII (APOC2) gene was localised to chromosome 19 and shown to be closely linked to APOE, the cDNA probe being found to detect a frequent RFLP (Myklebost 1984). Linkage analysis of APOC2 in DM families subsequently showed close linkage (Shaw *et al* 1985). The possibility of NF-1:DM linkage was therefore explored by studying the segregation of PEPD, APOC2, Lu, Se and C3 in NF-1 families.

2. Methods

A. Family ascertainment - The linkage family panel for the chromosome 19 studies was assembled prior to the population study. The families were ascertained through the records of the Section of Medical Genetics, University Hospital of Wales (families NF3, 7, 9, 12, 49, 71) and the British Neurofibromatosis Patients' Association (LINK, families NF70, 72, 73). The clinical assessment and diagnostic criteria applied were as described for the population study, apart from the classification of the "equivocal" status; for the linkage studies any child (<10 years) with 1-5 CAL spots and no other features was considered equivocal. From relevant family members a sample of venous blood (10-30 ml) was taken into tubes containing the anticoagulant EDTA for DNA, Lu and PEPD typing, and specimens of saliva were collected into sterile universal containers for Se typing.

B. Typing of C3 and APOC2 RFLPs - The methods used in this section (unless stated) are taken from Maniatis *et al* (1982). A diagrammatic summary of DNA analysis is shown in Figure 8-1.

(i) DNA extraction - The specimens of whole blood were stored at -20°C until DNA extraction could be performed; this was done using a modification of the method described by Kunkel *et al* (1977) as follows:

(a) The sample was thawed and mixed with sucrose lysis buffer (0.32M sucrose, 0.01M Tris-HCl pH 7.5, 1% Triton x 100, 0.005M MgCl), 90 or

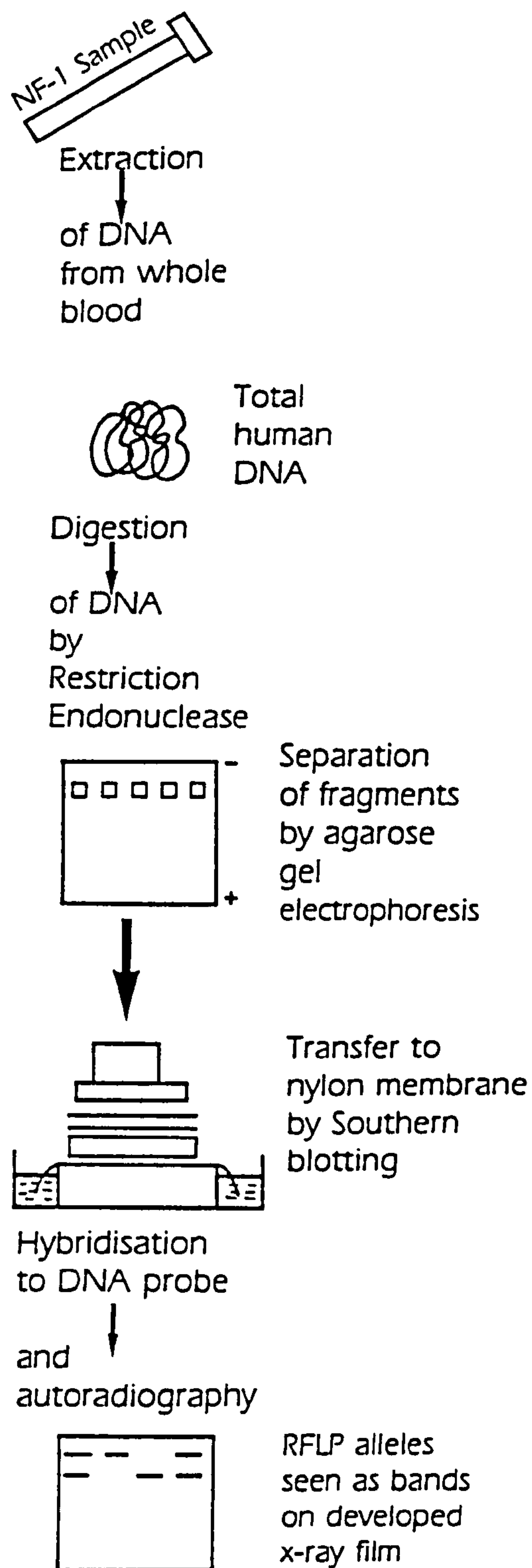


FIGURE 8-1. Diagrammatic representation of the procedures involved in RFLP analysis.

180 ml of buffer was used for 10 and 20 ml of blood respectively. The specimen was then centrifuged at 10 rpm for 20 minutes.

(b) The resulting nuclear pellet was then resuspended in 4.5ml of DNA extraction buffer (0.075M NaCl, 0.024M EDTA pH 8.0). 250µl of 10% sodium dodecyl sulphate (SDS) and 100µl of 10mg/ml proteinase K were then added. The mixture was then incubated at 55°C for 3 hours.

(c) 5ml of phenol (saturated with 20mM Tris-HCl pH 8.0) were added and the tube gently mixed, then spun at 10,000 rpm for 15 minutes.

(d) The upper aqueous layer was re-extracted with 2.5ml of chloroform-isoamyl alcohol mixture (24:1) and 2.5ml of phenol then centrifuged at 10,000 rpm for 5 minutes.

(e) The upper aqueous layer was re-extracted with 5ml of CHCl₃, and centrifuged at 10,000 rpm for 5 minutes.

(f) The DNA was precipitated from the aqueous phase by adding 2.5ml of 7.5M NaOAc and 10ml of 100% ethanol.

(g) The DNA pellet was placed in an eppendorf tube and dissolved in TE buffer (10mM Tris, 1mM EDTA pH 7.5).

(h) The DNA and protein concentrations were estimated by a spectrophotometric method (Schleif & Wensink 1981). DNA absorbs ultraviolet light so efficiently that optical absorbance at 260 nm gives an accurate and rapid measure of concentration. Absorbance at 280 nm gives the concentration of protein. A sample with a 260:280 ratio greater than 1.8 was accepted as being sufficiently pure to enable its digestion with restriction enzymes.

(ii) Preparation of APOC2 and C3 probes - The cDNA probe for the APOC2 gene was provided by Dr O Myklebost, who had previously reported that it identified a frequent Taq 1 polymorphism (Myklebost

et al 1984). The clone was prepared for use as a probe by Dr D Shaw who, after a standard plasmid preparation, had separated the coding sequences from the vector by restriction enzyme digestion to enhance the efficiency of radioactive labelling (Feinberg and Vogelstein 1984).

The human genomic C3 DNA probe was provided by Dr G Fey. This probe identifies a relatively frequent Sst1 polymorphism (Davies *et al* 1983). Dr L Meredith prepared the plasmid DNA for use by standard methods, satisfactory labelling was achieved without separation of the coding sequences from the vector.

(iii) Restriction enzyme digestion - The enzymes used were Taq 1 (APOC2) and Sst1 (C3) which require the same conditions to work. To each probe the 33 'key' individuals were first typed and then, for those informative (i.e.heterozygous), the relevant remaining family members were studied.

5 µg DNA digests were made up for each individual containing 2 µl core buffer, 2 µl bovine serum albumin (BSA), 1 µl 0.1M spermidine, 10 units of the appropriate restriction enzyme (i.e. 2 units/µg DNA) and the volume made up to 20 µl with distilled water. The digest was then incubated for 2 hours at 37°C. Any sample showing incomplete digestion on an electrophoresis checking gel was redigested with additional amounts of enzyme.

(iv) Electrophoresis - Following complete digestion of DNA samples by restriction enzymes, the DNA fragments were separated according to molecular weight by agarose gel electrophoresis.

Electrophoresis gels were made with 0.8% agarose dissolved in

electrophoresis buffer (4 mM Tris-acetate, 0.1 mM EDTA) with 0.5 µg/ml of ethidium bromide. The latter dye intercalates between DNA bases and allows visualisation of DNA on gels under ultra-violet light (Sharp *et al* 1973). After heating to dissolve the agarose, the cooled solution was poured onto a glass plate gel mould. Wells in the gel were formed using a comb with 20 teeth of 2mm width.

When set, the gel was placed in an electrophoresis tank containing electrophoresis buffer. DNA digest samples were mixed with ficoll-orange G to act as loading buffer and tracking dye, and introduced into individual slots in the gel. Electrophoresis was carried out overnight at 30v (70 MA). Visualisation of the gel under ultra-violet light confirmed that digestion and electrophoresis were complete.

(v) Southern blotting - In order to localise particular restriction fragments it is necessary to transfer the DNA from the gel onto a nitrocellulose or nylon filter before hybridisation to the DNA probe. This procedure was first described by Southern (1975) and preserves the relative position of the DNA fragments during transfer. Prior to blotting the double strands of DNA in the gel are denatured by alkali treatment to render them single stranded and therefore ready for subsequent hybridisation to the probe.

The gel was denatured in alkaline solution (0.5M NaOH, 1.5M NaCl) on an agitator for 90 minutes, and then neutralised in acidic solution (1.5M NaCl, 1M Tris pH 7.5) for 90 minutes, the solution being changed after the first hour. The DNA was then transferred to "Zetaprobe" nylon membrane (Amersham) by Southern blotting (Figure 8-2). A piece of 3MM Whatman paper was placed over a 'gel sized'

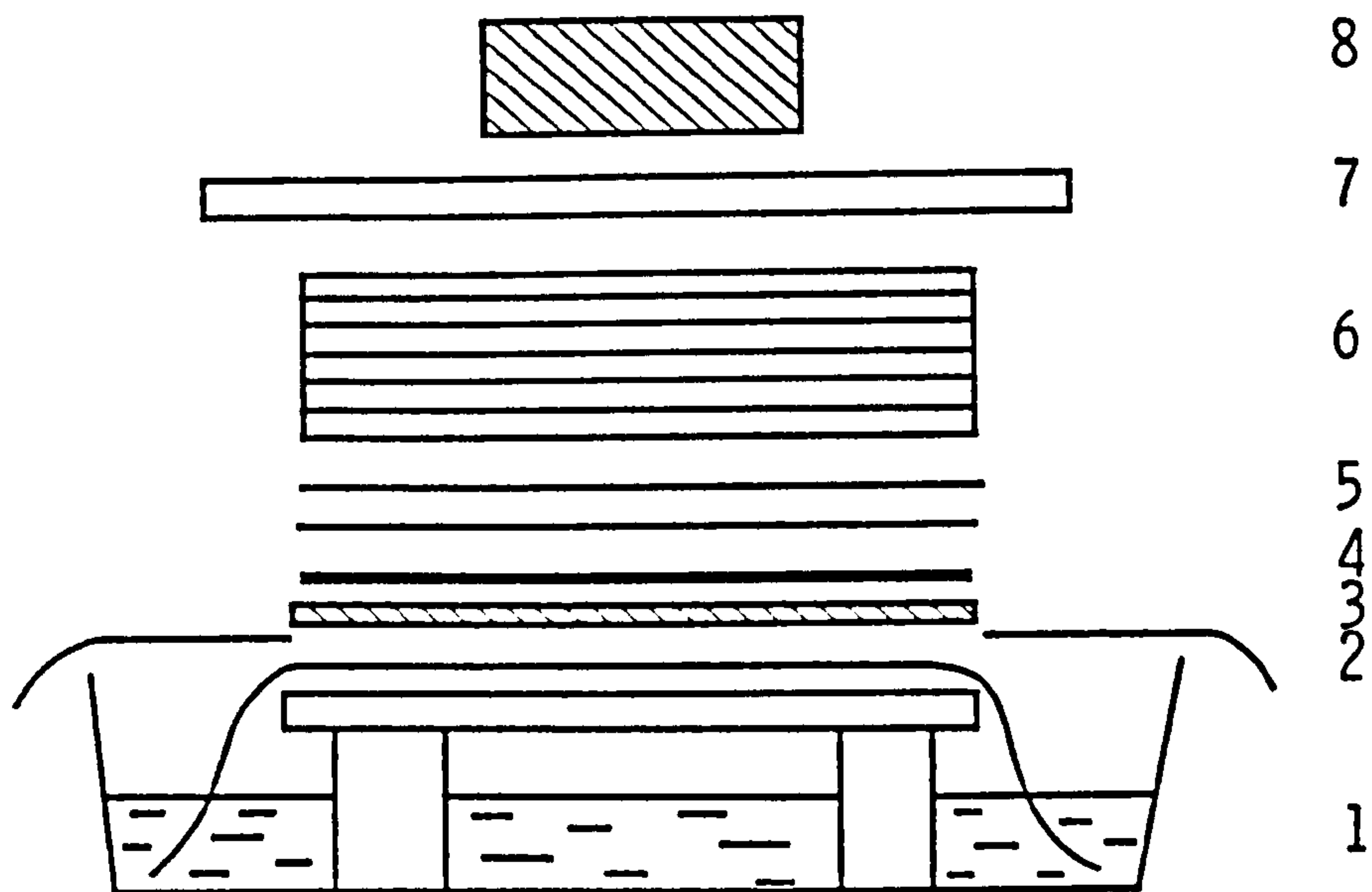


FIGURE 8-2. Southern blotting procedure. Shown in cross-section are: (1) tray containing 10xSSC, a glass plate (on supports) covered with Whatman 3MM paper presoaked in 10xSSC; (2) cling film cover around gel edge; (3) agarose gel; (4) membrane; (5) 2 sheets of Whatman 3MM paper presoaked in 10xSSC; (6) paper towels; (7) glass plate and (8) weight.

glass support and dipping into a 10 x SSC reservoir (prepared from stock solution of 20 x SSC, 3M NaCl and 0.3M NaCit). The gel was placed on top of this, and the edges sealed to the edge of the container using cling-film. The gel was then covered with a piece of Zetaprobe presoaked in 10 x SSC. Two pieces of 3MM Whatman paper also soaked in 10 x SSC were placed on top and covered with a stack of absorbent paper towels. A glass plate and weight were placed on top of the towels. This was then left overnight. Transfer occurs because as liquid flows from the reservoir upwards, the DNA is eluted from the gel and deposited onto the membrane. The procedure is shown in Figure 8-2.

After transfer, the membrane was washed in 2 x SSC and baked for 3 hours at 80°C.

(vi) Nick translation of probe DNA - The DNA probes were radioactively labelled using the nick translation process (Balmain & Birnie 1979). This is catalysed by *E. coli* DNA polymerase I which adds nucleotide residues to the 3'-hydroxyl terminus that is created when one strand of a double-stranded DNA molecule is nicked by the action of DNase I. In addition, the enzyme, by virtue of its 5' to 3' exonucleolytic activity, can remove nucleotides from the 5' side of the nick. Nucleotides are therefore added to the 3'-hydroxyl side of the nick and removed from the 5' side, the nick is thus 'translated' along the DNA. By replacing existing nucleotides with radioactive ones the DNA becomes labelled.

The DNA probes were radiolabelled using a nick translation kit (Amersham). 5 µg probe DNA was mixed with 10 µl nucleotide buffer, 5 µl of enzyme solution (DNase 1 and polymerase 1), 2.5 µl of

deoxycytidine triphosphate labelled with radioactive phosphorus (α - ^{32}P dCTP) and distilled water to a total volume of 50 μl . The mixture was incubated at 16°C for 2 hours. Labelled DNA was then separated by passage of the mixture through a Sephadex G-50 column suspended in elution buffer (150 mM NaCl, 10 mM EDTA, 0.1% SDS, 50mM Tris HCl pH 7.5). 100 μl aliquots were collected into 20 Eppendorfs by adding elution buffer to the top of the column, and monitored for radioactivity by scintillation β counting. The peak fractions containing labelled DNA were pooled; specific activity of the probe DNA of 1×10^7 cpm/0.1 μg or greater was considered sufficient for hybridisation experiments. The labelled DNA was boiled for 5 minutes and then cooled in ice to dissociate the DNA molecules into single strands.

(vii) Hybridisation - By this process the single strand probe DNA joins with homologous sequences on the membrane. The membrane was prepared for hybridisation by soaking initially in 0.1 x SSC and 0.5% SDS for 1 hour at 65°C and then in a solution of 5 x SSC, 10 x Denhardt's solution (2% Ficoll, 2% BSA, 2% polyvinyl pyrrolidane), 0.5 mg/ml herring sperm DNA and 50mM phosphate (pH 6.5) for 3 hours at 65°C.

The membrane was then hybridised with the probe overnight at 65°C in a solution of 5 x SSC, 2 x Denhardt's solution, 100 $\mu\text{g/ml}$ herring sperm DNA and 10% dextran sulphate. After hybridisation the membrane was then washed in 4 x SSC and 0.1% SDS 3 times for 15 minute periods at 65°C. If high background activity was still detected with a hand-held β counter, the filters were washed in solutions of increasing stringency at 65°C for 15 minutes, initially 1 x SSC and

0.1% SDS was used and then, if necessary, 0.5 SSC and 0.1% SDS.

(viii) Autoradiography - The fragments of sample DNA to which the probe had hybridised were then visualised by autoradiography. The filters were then wrapped in cling film and autoradiographed in cassettes with intensifying screens at -70°C. The film was developed after 24 hours; if the band intensity was insufficient to allow interpretation, the filters were exposed to new X-ray film for longer periods (2-7 days).

C. Secretor typing -

(i) Background - The A, B and H blood group antigens are not confined to the red cells but are widely distributed throughout the body. Yamakami (1926) noted that the antigens were present in saliva, and in 1930 Lehrs and Putkonen realised independently that some individuals secreted the antigens in saliva and others did not. The ability to secrete the A, B or H antigen in the saliva was shown by Schiff and Sasaki to be a Mendelian dominant in 1932. Secretor status is independent of ABO blood group and the locus was shown to be linked to the Lu blood group by Mohr in 1954. Although the ability to secrete the antigens is independent of the blood groups, in secretors the antigens present in saliva reflect the blood group as shown in table 8-1 below.

Table 8-1 Expected ABH antigens in saliva

ANTIGEN IN SALIVA			
ABO group secretors	A	B	H
A	Much	None	Less
B	None	Much	Less
O	None	None	Much
AB	Much	Much	Less
Non-secretors (regardless of blood group)	None	None	None

(ii) Method - The method of secretor typing used was taken from Race and Sanger (1975).

(a) On the day of collection the saliva was boiled for 10 minutes to destroy enzyme activity and break down muco-proteins. The specimen was then centrifuged (bench centrifuge, maximum speed, 5 minutes) and the clear supernatant fluid removed and stored at -20°C until tested.

(b) The antisera for the A, B and H antigens were obtained from Biotest Folex Ltd.. They were titrated against appropriate red cells (doubling dilutions for anti-A and B, serial dilutions for anti-H). The dilutions used for the subsequent test were the last but one to give good agglutination: 1/16 for anti-A and anti-B and 1/6 for anti-H.

(c) The test saliva was diluted 1:2 with normal saline. To one

volume of dilute saliva in 3 separate tubes one volume of anti-A, anti-B and anti-H was added. After 20 minutes, a volume of 2% A₂, B and O cells were added to the appropriate tubes (A₂ cells are a more sensitive test of inhibition than A₁); the cells had been washed with saline 4 times to wash off A, B, H substances in the plasma and diluted to an approximately 2% suspension by eye.

(d) After one hour the results were read based on the presence/absence of agglutination as shown in Table 8-2 below.

Table 8-2 Agglutination results for secretors of blood group
O, A, B and AB, and non-secretors

	Antigen present		
	Anti-A	Anti-B	Anti-H
Non-secretor	+	+	+
O-secretor	+	+	0
A-secretor	0*	+	0
B-secretor	+	0	0
AB-secretor	0	0	0

* + indicates agglutination present; 0 : no agglutination.

D. Lu and PEPD typing - The Lu blood group and PEPD typing was done in the laboratories of Dr P Tippett and Professor E B Robson, respectively .

E. Linkage analysis - The linkage analysis was performed using the programme LIPED 3 (Ott 1974) with the assistance of Dr M Sarfarazi. In the case of unaffected relatives, only those over the age of 5 years were included because of the uncertainty about the age by which affected subjects will have manifested CAL spots. Penetrance for the NF-1 gene was taken to be 100%. The gene frequencies for secretor status were taken from Race & Sanger (1975, secretors 0.77, non-secretors 0.23).

3. Results

Fifty-seven affected subjects and 41 normal relatives from the 9 families were studied. Three children were excluded because of equivocal clinical findings (NF7, IV-11; NF72, IV-2; NF73, IV-7); patient IV-5 in family NF7 was also equivocal but he and his two unaffected siblings (IV-2,3) were also excluded on the grounds of possible non-paternity.

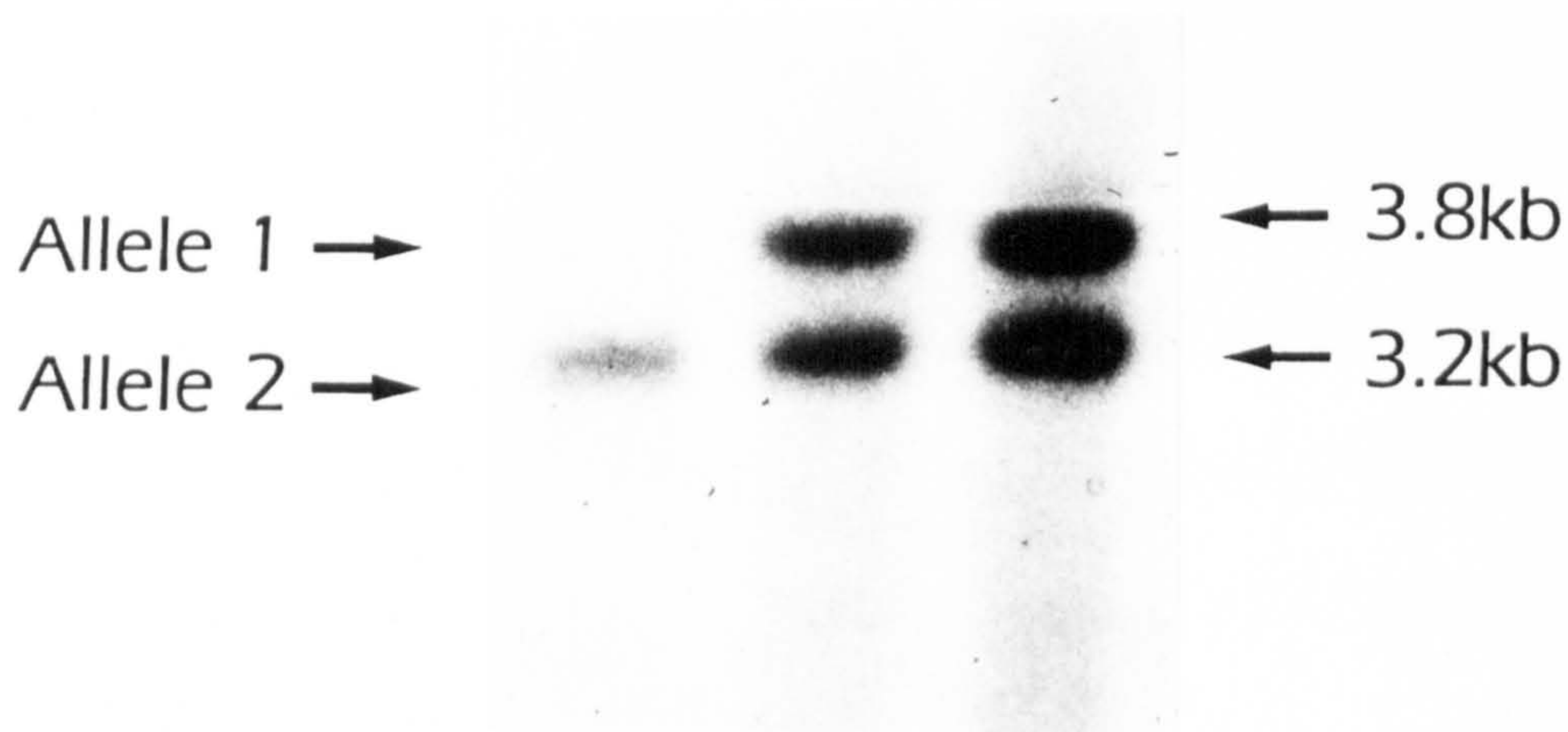
The Taq 1 polymorphism detected by the APOC2 probe is a 2 allele system with alleles of lengths 3.8 kb (allele 1) and 3.2 kb (allele 2). The allele frequencies in the population studied were 0.56 (allele 1) and 0.44 (allele 2). This polymorphism is illustrated in Figure 8-3a.

The Sst 1 polymorphism detected by the C3 probe is shown in Figure 8-3b. It consists of 2 alleles, a single 12.0 kb band (allele 1) or 2 bands (9.0 and 3.0 kb, allele 2). The frequencies in the population studied were 0.53 and 0.47 respectively.

None of the families were informative for Lu or PEPD.

Taq 1 Polymorphism (APOC2)

(A)



Sst 1 Polymorphism (C3)

(B)

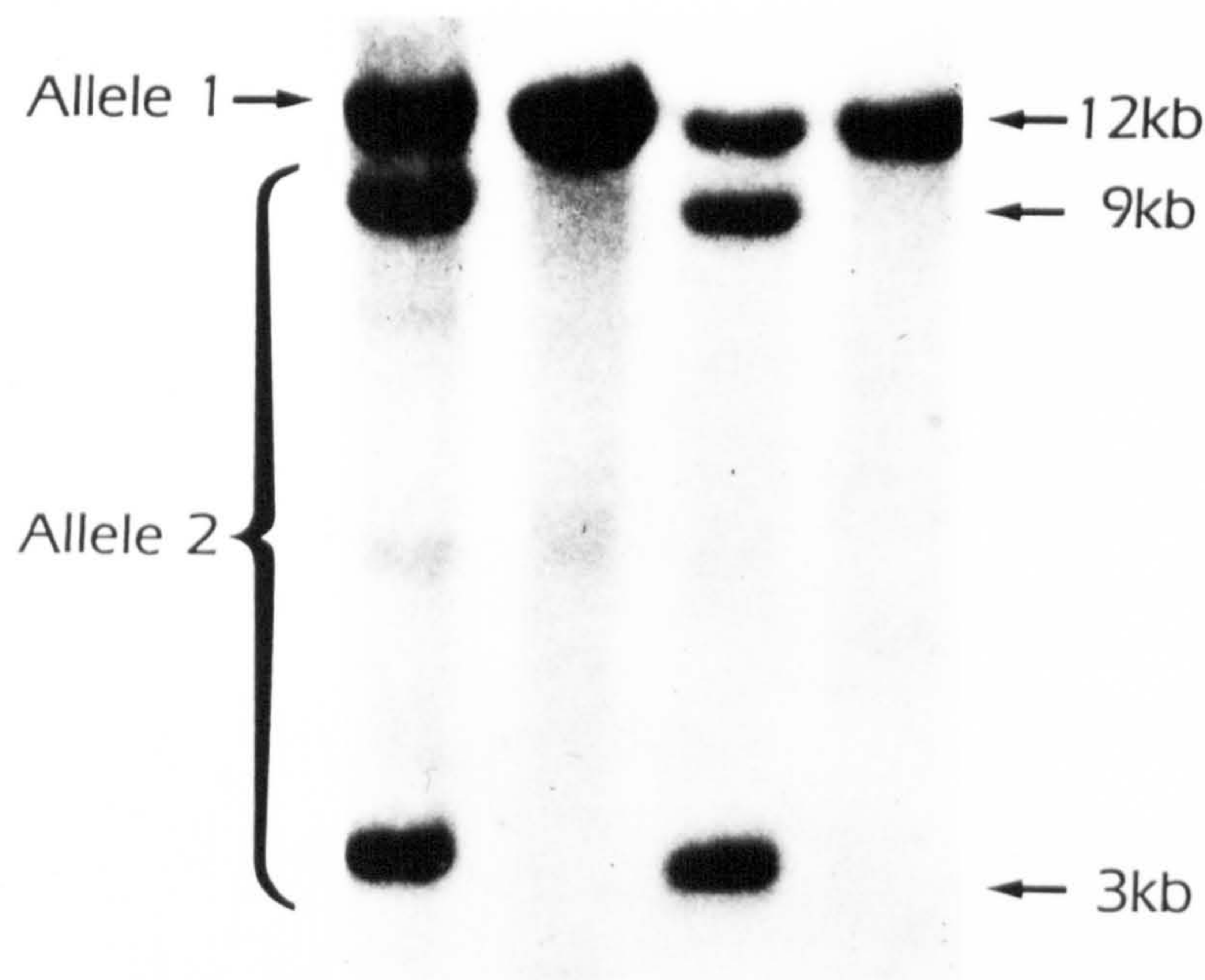


FIGURE 8-3. APOC2 (Taq 1) and C3 (Sst 1) polymorphisms.

Table 8-3 Lod scores between NF-1 and APOC2, PEPD, Se

Marker	Lod score	No. of meioses studied	Recombination fraction				
			0.01	0.05	0.10	0.20	0.30 0.40
APOC2	Males	10	<u>-8.40</u>	-4.62	-3.00	-1.53	-0.81 -0.38
	Females	27	<u>-11.09</u>	-7.29	-4.51	-2.02	-0.88 -0.32
	Assuming						
	$\theta_M=\theta_F$		<u>-22.63</u>	-11.85	-7.42	-3.48	-1.65 -0.70
C3	Males	7	<u>0.26</u>	0.20	0.14	0.05	0.00 0.00
	Females	10	<u>-3.39</u>	-1.95	-1.31	-0.68	-0.33 -0.12
	Assuming						
	$\theta_M=\theta_F$		<u>-3.78</u>	-1.82	-1.09	-0.51	-0.26 -0.11
Se	Males	6	<u>-1.58</u>	-0.88	-0.57	-0.27	-0.11 -0.03
	Females	15	<u>-2.60</u>	-1.24	-0.70	-0.29	-0.07 -0.02
	Assuming						
	$\theta_M=\theta_F$		<u>-4.12</u>	-2.08	-1.25	-0.52	-0.19 -0.05

The results of linkage analysis are shown in Table 8-3 and the typing results shown on the pedigrees in Appendix B. The three informative markers all showed significant evidence against linkage ($\text{lod} \leq -2.0$) for at least some values of θ . There was no evidence of linkage when male and female meioses were analysed separately. No family showed a positive result which might have suggested non-allelic heterogeneity.

These results were published in the paper of Huson *et al* (1986).

4. Conclusions

The physical map and genetic relationships of the markers studied are shown in Figure 8-4. The information used is taken from Shaw *et al* (1986) and Naylor *et al* (1985). APOC2 is the closest marker to DM with a maximum lod score of 23.9 at $\theta = 0.04$; C3 and Se flank the DM locus but are much less closely linked. This study showed clear evidence against linkage with all 3 informative markers and therefore excluded the possibility of linkage of NF-1 to DM and the NF-1 locus from a considerable region of chromosome 19.

In view of the negative findings, an explanation had to be found for the apparent co-segregation of NF-1 and DM in the families reported by Ichikawa *et al* (1981) and Rivas and Di Liberti (1984). One explanation would be that there is more than one NF-1 locus; another that the affected individuals in these families actually have a disease in which the features of NF-1 and DM are combined. It was felt that both these possibilities were unlikely and that the explanation is that the clinical assumptions that have to be made to show linkage in these families were incorrect.

The family reported by Ichikawa *et al* (1981) is the only one for which full clinical details have been published. In this family, the origin of NF-1 in generation I is clearly from the mother but that of DM is uncertain. If her husband had DM then there is no evidence of linkage in this family. This would be supported by the fact that in generation IV (not included in the linkage analysis) there is an individual who has congenital DM and no stigmata of NF-1 at the age of 2 years. As shown in the clinical studies in this thesis, this would be very unusual if he had inherited the NF-1 gene.

This work was later confirmed and extended by Pericak-Vance *et al* (1987) who, in addition to finding no evidence of linkage with APOC2 and C3, also found no linkage with locus D19S6 on the long arm of chromosome 19. NF-1 was therefore virtually excluded from this chromosome.

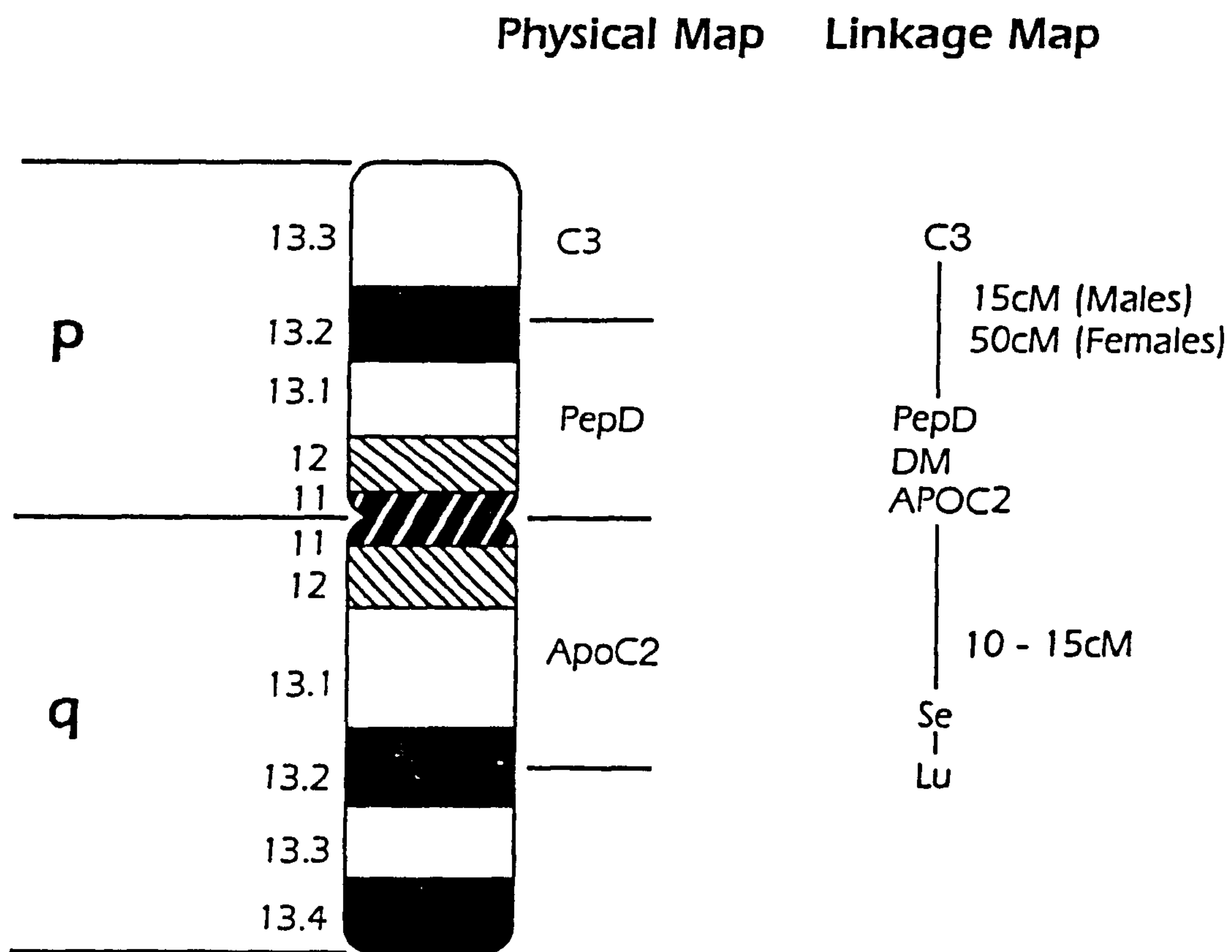


FIGURE 8-4. Physical and genetic map of chromosome 19.

CHAPTER 9 An exclusion map for NF-1

1. Introduction

With the exclusion of linkage of NF-1 to the DM region of chromosome 19, one of the principal 'clues' to the localisation of the NF-1 gene had been disproved. It therefore seemed likely that a 'search' of the genome was going to be necessary to map the gene. The author's main emphasis at this time was on the population study in South East Wales; linkage analysis of other markers using the family panel described in Chapter 8 was continued in Cardiff and samples from the family panel were made available to collaborators in other centres with a view to expediting the mapping of the gene. As families with a suitable structure for linkage analysis (and who were agreeable to giving samples) were identified through the population study, blood samples for DNA analysis were collected (NF-1, 17, 20, 41 and 42).

In this chapter the results of linkage analysis of other markers in the family panel are presented. By mid-1986, with further negative data from markers on other chromosomes, the author compiled the available linkage data for an exclusion map of NF-1; this is presented in the final section.

2. Outcome of linkage analysis of other genetic markers and NF-1

The results of the analysis of the markers described in sections (A) and (B) are presented in Table 9-1.

(A) Protein polymorphisms - In addition to typing the families for PEPD, the MRC Human Biochemical Genetics Unit (courtesy of Professor E B Robson and Dr S Povey) also typed 4 protein polymorphisms: Group-specific component (Gc), immunoglobulin gamma 1 polypeptide (Gm), alpha-1-antitrypsin (Pi) and the sixth component of complement (C6). The results all showed significantly negative lod scores. The Gc results showed no evidence of heterogeneity as had been suggested by the earlier results of Spence *et al* (1983).

(B) Studies of other DNA polymorphisms - The typing of further DNA polymorphisms in Cardiff was done by Dr M Upadhyaya. The strategy chosen was to systematically exclude the gene from given chromosomes using highly polymorphic DNA probes available in the laboratory and from various colleagues. The only specific 'clue' followed arose from the probable assignment of NF-2 to chromosome 22 by the work of Seizinger and colleagues in 1986 (Seizinger *et al* 1986a). They demonstrated loss of chromosome 22 markers in both isolated acoustic neuromas and those from patients with NF-2. No evidence of linkage was found with the IGLV probe, and this, taken with the previously published negative results for the locus SIS (Seizinger *et al* 1986b) showed that NF-1 and NF-2 were genotypically as well as phenotypically distinct.

The negative results with 3 chromosome 20 markers, two on 20p and one on 20q, excluded NF-1 from this chromosome in view of its small size.

Table 9-1 Linkage analysis between NF-1 and marker loci discussed in Sections 9-2 A and B

Genetic marker	HMG8	Recombination fraction (θ), assuming $\theta_M=\theta_F$					
	localisation						
		0.01	0.05	0.10	0.20	0.30	0.40

Protein polymorphisms
(9-2A)

Gc	4q12-q13	<u>-5.07</u>	-2.31	-1.11	-0.19	-0.08	0.09
Gm	14q32.3	<u>-2.89</u>	-0.96	-0.27	0.13	0.13	0.04
Pi	14q32.1	<u>-7.89</u>	-4.52	-3.10	-1.69	-0.91	-0.41
C6	Unassigned	<u>-8.00</u>	-3.97	-2.35	-0.95	-0.34	-0.08

DNA polymorphisms
(9-2B)

Met D	7q22-q32	<u>-2.43</u>	-1.17	-0.58	-0.12	0.03	0.05
α globin	16pter-p12	<u>-4.49</u>	-1.93	-0.74	0.12	0.27	0.16
D 20S6	20p	<u>-7.66</u>	-3.78	-1.93	-0.49	-0.06	0.00
D 20S5	20p12	<u>-6.79</u>	-2.75	-1.20	-0.09	0.15	0.08
D 20S4	20q13.2	<u>-10.44</u>	-5.12	-3.03	-1.26	-0.53	-0.19
IGLV	22q11.1-q11.2	<u>-15.82</u>	-7.56	-4.13	-1.33	-0.31	-0.02

(C) Collaboration with other Centres

(i) Studies of β Nerve Growth Factor (β -NGF) - The early reports of abnormalities of NGF levels in NF-1 patients are reviewed in Chapter 1. The cloning of the gene for β -NGF (Ullrich *et al* 1983) meant that since it was found to detect RFLPs (Breakefield *et al* 1983; Darby *et al* 1985) it could be directly tested as a candidate gene by studying the segregation of these RFLPs in NF-1 families. In an initial report in 1985, Darby and his colleagues reported several recombinants with a lod score of -3.77 at $\theta = 0.05$ in 4 families with NF-1. Although this made it unlikely that β -NGF was the candidate gene it was felt more families should be tested to exclude heterogeneity. DNA samples from our original 9 families were therefore sent to Dr Darby. Again, multiple recombinants were found with a combined total lod score of -17.2 at $\theta = 0.01$ (Darby *et al* 1986). No family gave a positive result suggesting heterogeneity.

(ii) Other candidate genes - The laboratory of Dr J Gusella was testing other possible candidate genes (oncogenes, growth factors and their receptors) for NF-1 by the linkage approach. Specimens from the 5 largest families (NF-7, 12, 49, 72, 73) were therefore sent to Dr Gusella so that lymphoblastoid cell lines could be established and the families used in their work. The results were all negative and were published in the paper of Seizinger *et al* (1987b).

(iii) Hypervariable minisatellite probes - Hypervariable minisatellite regions, consisting of tandem repeats of a short sequence and showing multiallelic variation in repeat copy number,

exist in human DNA and provide highly informative genetic markers. In 1985 Jeffreys *et al* (1985a & b) reported the development of two probes consisting of tandem repeats of the "core" sequence of one subset of human minisatellites. The two probes each detect a different pattern of variable DNA fragments to produce distinct DNA 'fingerprints' that are individual-specific and show somatic and germ-line stability.

The probes can be used in linkage analysis if particularly large sibships are available and up to 34 hypervariable loci analysed simultaneously (Jeffreys *et al* 1986). If a locus is identified that apparently segregates with the disease, it can be isolated and locus-specific probes developed for further analysis.

Dr Jeffreys kindly agreed to analyse family NF-49 with probes 33.6 and 33.15 and no locus of potential interest was identified.

3. An exclusion map for NF-1

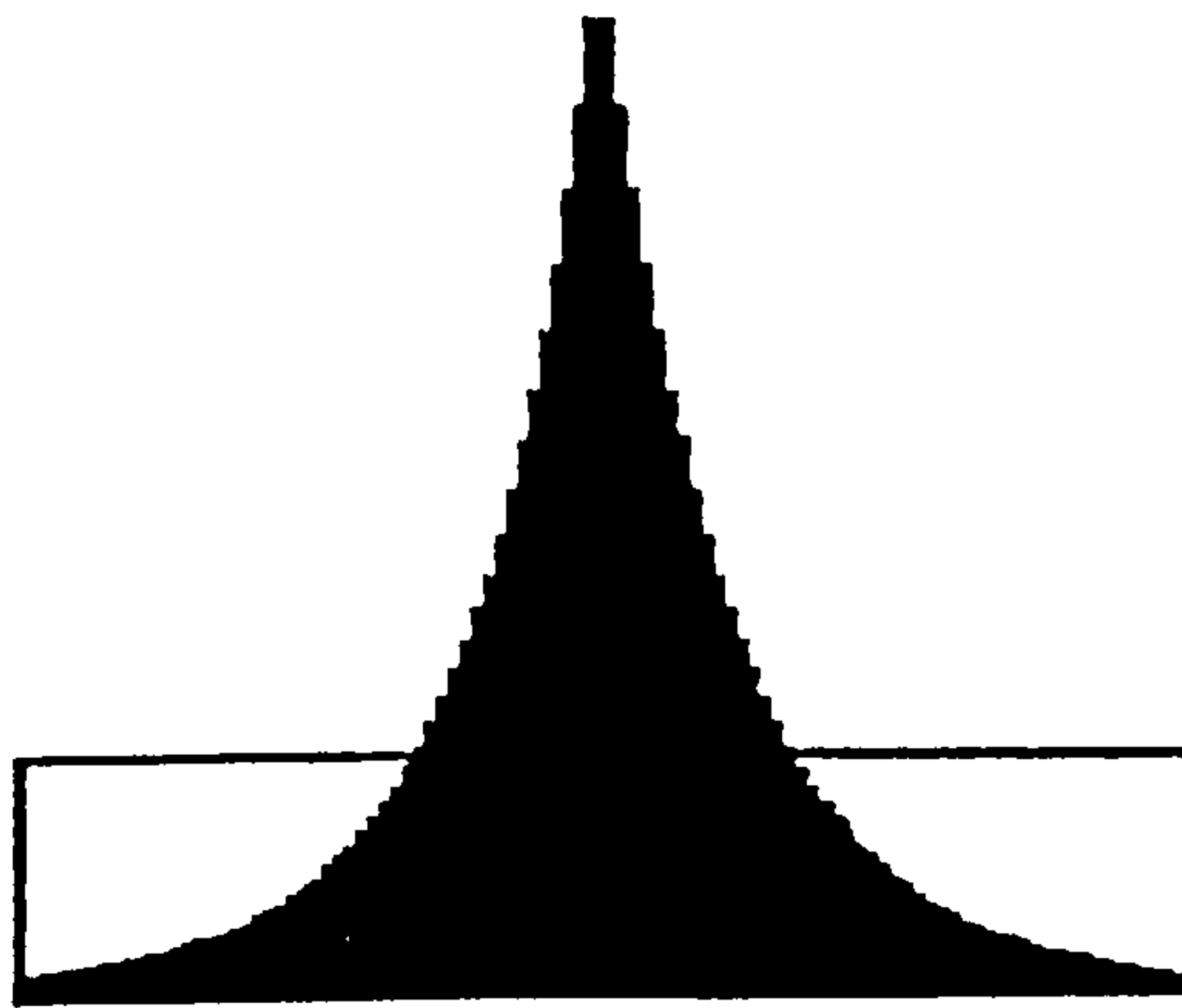
(A) Background - By 1986 the possible clues to the localisation of NF-1 had all been excluded; it was therefore apparent that a systematic search with RFLPs was going to be necessary to map the gene. As a prerequisite to this, it was necessary to gather all the available data in an 'exclusion' map, which would avoid unnecessary duplication of negative results and highlight those areas of the genome not yet studied. The author therefore tabulated the published linkage data with those from the Cardiff laboratory, and Dr M Sarfarazi used the program 'EXCLUDE' (kindly made available by Professor J H Edwards) to produce an exclusion map for NF-1.

(B) Description of the 'EXCLUDE' program - The program is designed to calculate the likelihood distribution of an unplaced locus around a series of previously mapped loci (Edwards 1987), following the general principle of Cook *et al* (1980) who first used the term 'exclusion mapping'. It is written in Pascal (Turbo version). The likelihood distribution at various recombination fractions or map distances gives the relative chance that the locus is in that position. When direct counts of recombinants and non-recombinants are available, the peak and position of the likelihood distribution are uniquely defined by them. As data are more usually presented as lod scores, the EXCLUDE program converts the data into equivalent observations, by defining the numbers of recombinants and non-recombinants which would give the same height and position of the likelihood distribution. For a given value of θ and the corresponding value of lod score (z), the equivalent number of informative meioses (s) is defined as :

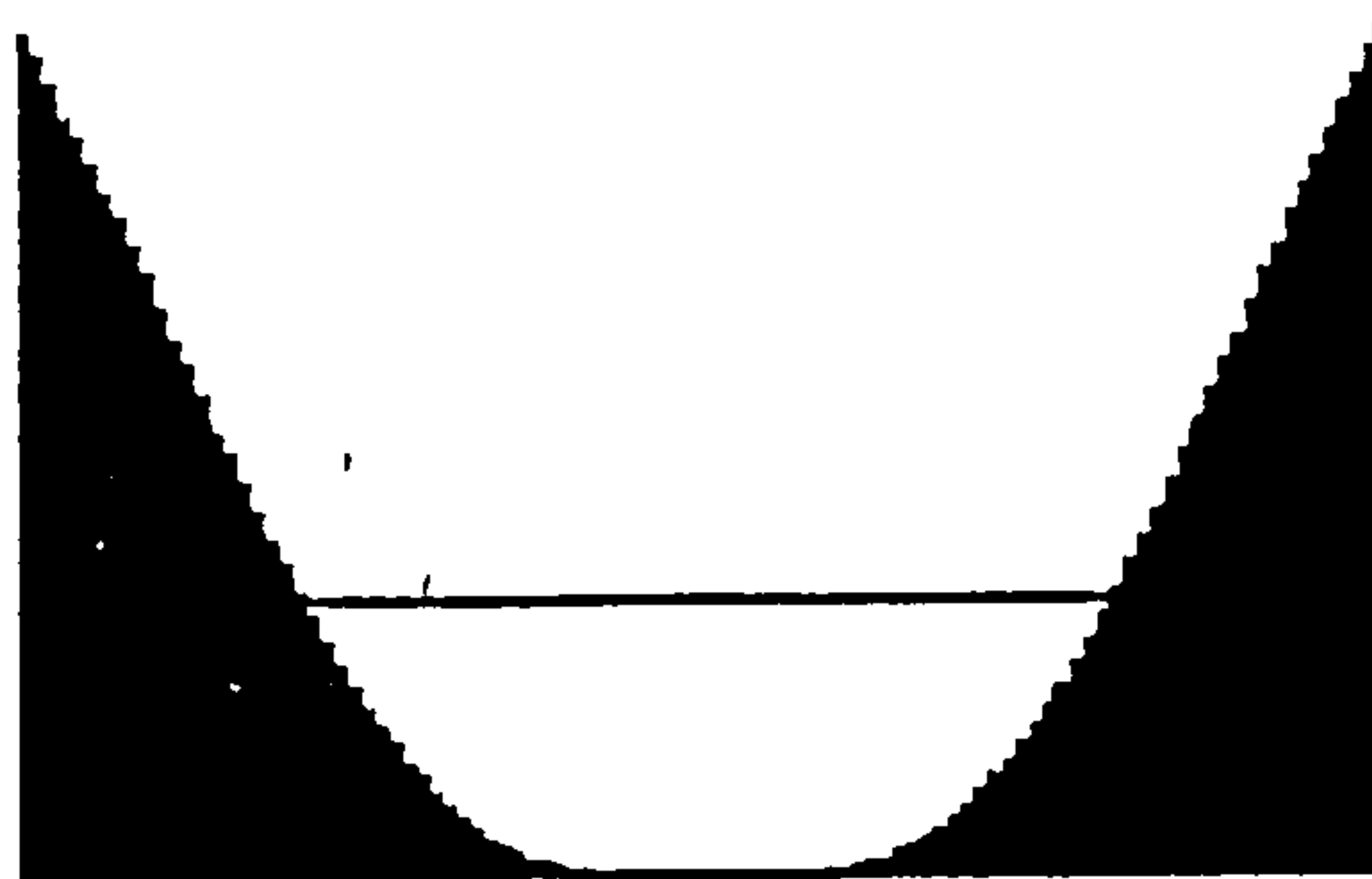
$$s = z / [\theta \cdot \log(2\theta + 1 - \theta) \cdot \log_2(1 - \theta)]$$

and the equivalent number of recombinants (r) = $\theta \cdot s$. The likelihood for each position is then computed directly from these equivalents.

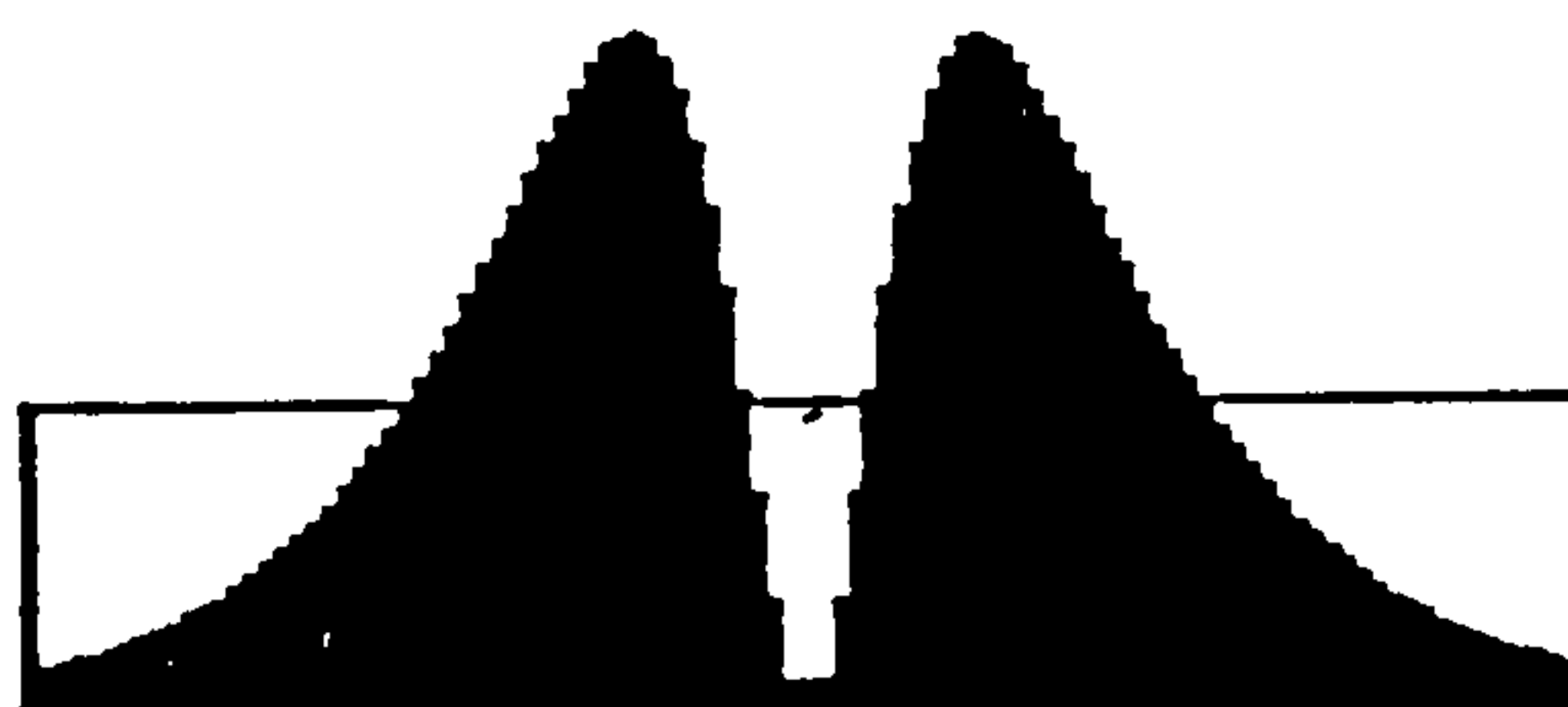
The input to the program consists of the locus name and position expressed as a percentage from pter-qter. When the localisation of a marker is expanded over a chromosomal region, the localisation is taken to be in the middle of that region. This is followed by the data, either in lod scores (in which case the program calculates the value of r and s) or recombinants and non-recombinants. For data which are uniformly negative, the lod score at $\theta = 1.10$ is usually used, with positive scores the maximum lod score and recombination at which it occurred are used. The program then accesses an internal



*Likelihood distribution for r:n of 0:10.
Chromosome length 100 cM. Marker locus at 50 cM.*



Likelihood distribution for r:n of 5:5. Chromosome length 100 cM. Marker locus at 50 cM.



*Likelihood distribution for r:n of 2:18.
Chromosome length 100 cM. Marker locus at 50 cM.*

FIGURE 9-1. Example of the likelihood distributions produced by the programme 'EXCLUDE' when a marker locus at 50 cM on a chromosome 100 cM length is studied with varying results. Reproduced from Edwards (1987) with permission.

file (HUMAN.CHR) which contains the approximate length of each of the human chromosomes in centimorgans. The program then calculates the positional likelihood of the disease locus on each chromosome, and also the percentage probability of any locus being on any of the 22 autosomes. The program presents a graphical display of the likelihoods of the locus being on a given chromosome. Examples are shown in Figure 9-1 to demonstrate this; these are taken from the paper of Edwards (1987, with permission of Professor Edwards and the publisher).

(C) Origin of linkage data - The data used for the exclusion map, in addition to those presented in Tables 8-3 and 9-1, are those published or presented before the middle of 1986 and are from the papers of Darby *et al* (1986), Dietz *et al* (1985), Dunn *et al* (1985), Lepage *et al* (1981), Seizinger *et al* (1986b) and Spence *et al* (1983, 1986). The data are presented in Table 9-2.

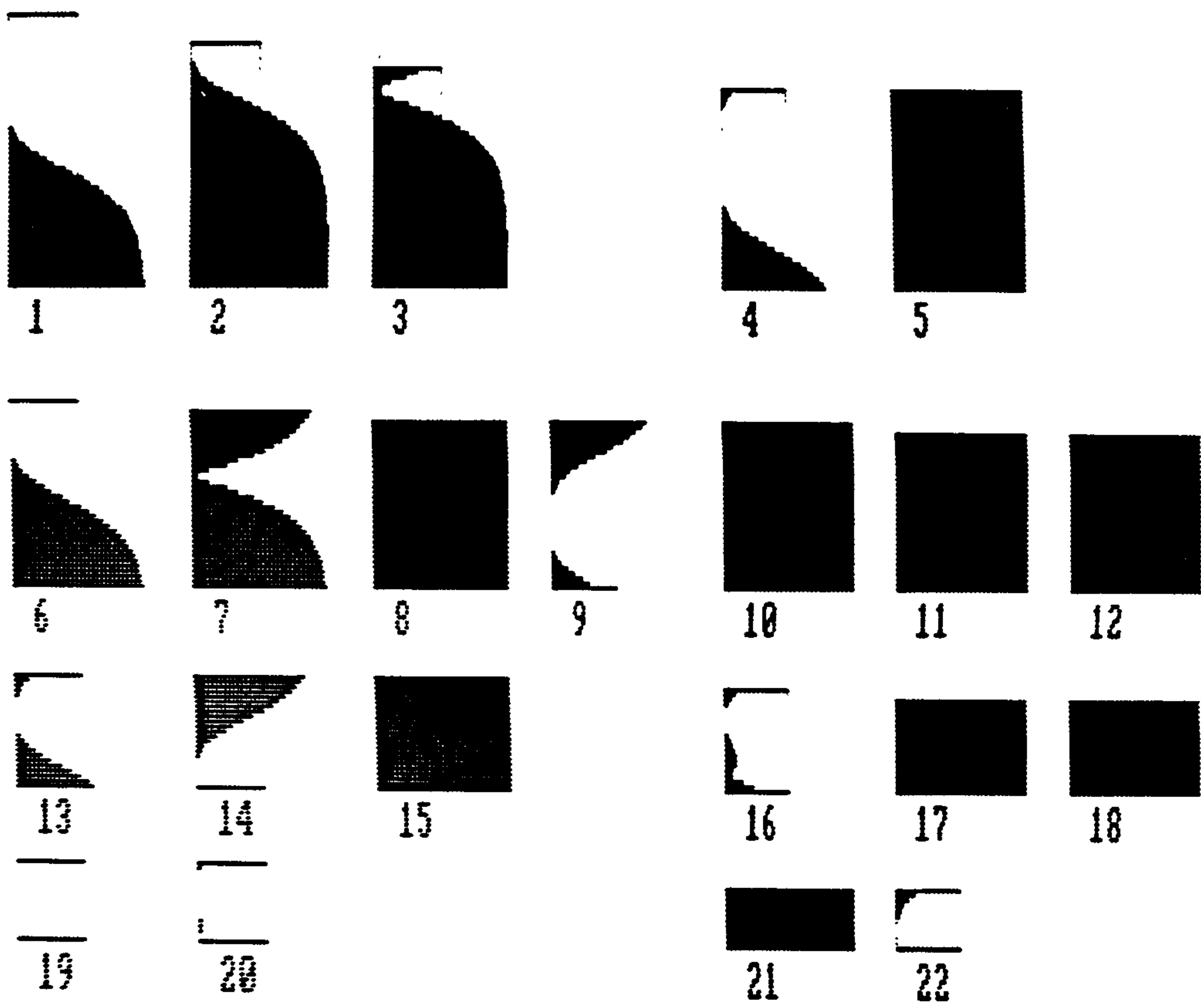
(D) The exclusion map for NF-1 - The exclusion map is presented in Figure 9-2a. It can be seen that no markers had been tested on 8 chromosomes but NF-1 had effectively been excluded from chromosomes 16, 19, 20 and 22, and in addition, from the majority of chromosomes 1, 4 and 9. This map was subsequently presented at the American Society of Human Genetics meeting in October 1986 (Upadhyaya *et al*), when other groups expressed an interest in pooling their linkage data to further extend the map. All groups involved in linkage analysis of NF-1 were subsequently invited to attend the first European Symposium on Neurofibromatosis (sponsored by LINK) in February 1987; the author was one of the principal organisers of this meeting. As a

Table 9-2 Combined linkage data for NF-1 used for exclusion map
shown in Figure 9-2

Marker	HGM8 localisation	LOD score $\theta = 0.10$	Source(s) of data*
RH	1p36.2-34	-10.4	3,8
DNF15S1	1p36	-1.1	1
PGM1	1p22.1	-2.9	3,8
NGF β	1p22.1	-4.8	1
FY	1p21-q23	-11.0	3,8
ACP1	2p25 or 2p23	-5.1	3,8
DNF15S2	3p21	-0.71	3
Gc	4q12-13	-11.4	2,3,8,9
MNSs	4q28-q31	-11.1	2,3,8
HLA	6p21.3	-6.7	4,7
GLO1	6p21.31-p21.1	-3.5	3,7
METD	7q22-32	-0.6	9
ABO	9q34	-7.1	3,7
ESD	13q14.1	-3.3	3,7
Pi	14q32.1	-3.1	9
Gm	14q32.3	-0.3	9
PGP	16pter-p11	-2.8	3,7
HBA	16pter-p12	-0.7	9
HP	16q22.1	-2.7	3,7
C3	19p13.3-13.2	-1.9	3,9
APOC2	19CEN-q13.2	-7.4	9
SE	19q	-2.3	3,9
D20S5	20p12	-1.2	9
D20S6	20p	-1.9	9
D20S4	20q13.2	-3.0	9
IGLV	22pter-q11	-4.1	9
SIS	22q12-13	-0.6	6

* The references numbered refer to: Darley *et al* 1986(1), Dietz *et al* 1985(2), Dunn *et al* 1985(3), Lepage *et al* 1981(4), Seizinger *et al* 1986(6), Spence *et al* 1983(7) & 1986(8), data presented in this thesis(9).

(A)



(B)

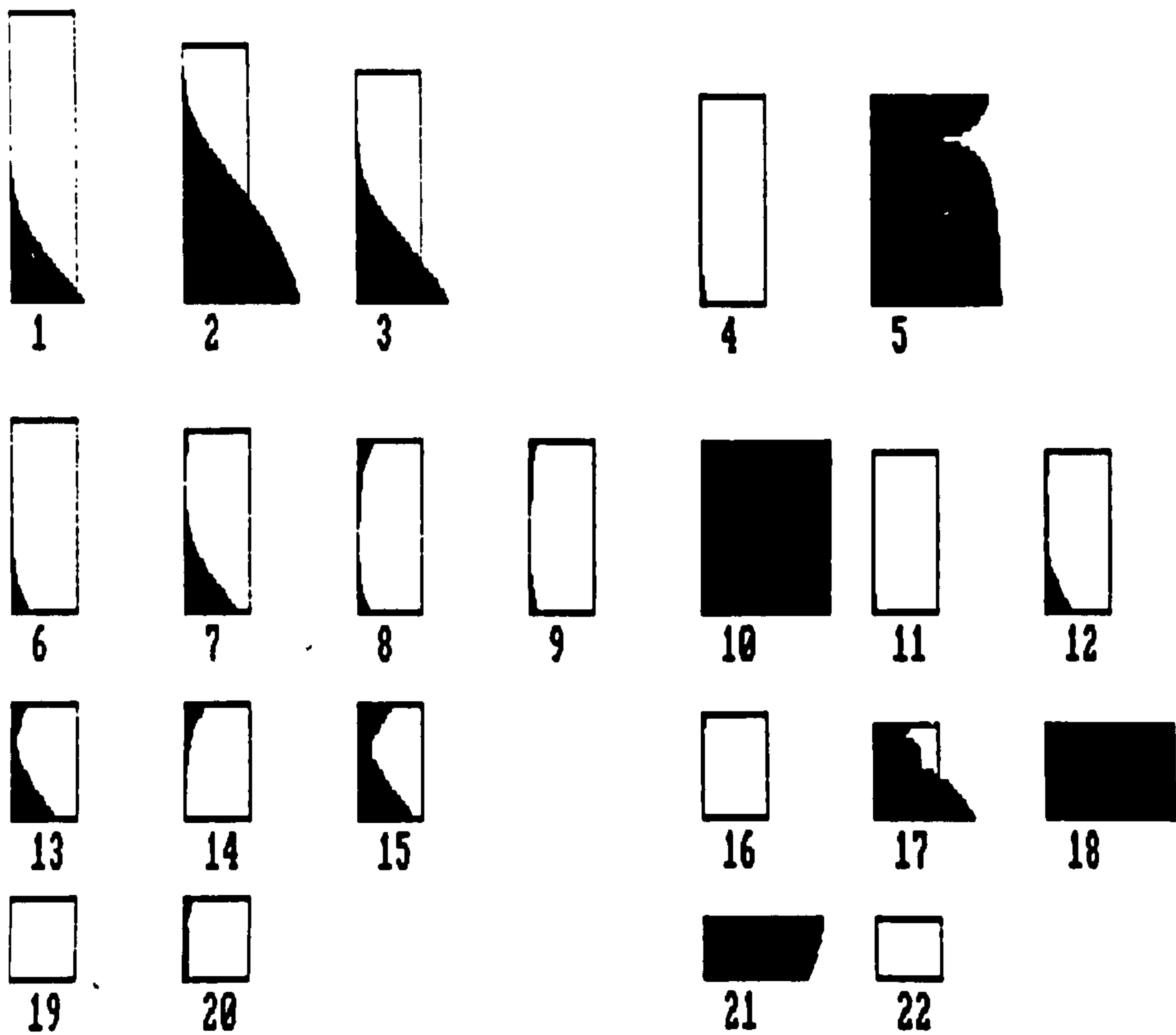


FIGURE 9-2. Exclusion map for NF-1. (A) using limited data set; (B) final exclusion map using pooled data of NF-1 linkage consortium.

result of this, the International Consortium on NF-1 Linkage was formed; its first task was to produce an updated exclusion map. The data were compiled by Dr M Sarfarazi and subsequently published (Sarfarazi *et al* 1987) in conjunction with a series of papers from the individual groups who had pooled data (*Journal of Medical Genetics*, 24, 522-538, 1987). 114 markers had been tested, for 90 of which the localisation was known and could therefore be used in EXCLUDE. The resulting map is shown in Figure 9-2b, and showed that the gene was most likely to be on chromosome 5, 10, 17 or 18. A small positive result for a marker on chromosome 17 (Barker *et al* 1987a) taken with the exclusion data, meant that this was the most likely localisation, with a likelihood seven times greater than for any other chromosome.

(E) Mapping of the gene for NF-1 - The final exclusion map was made available to Consortium members in March 1987 and within a few weeks the NF-1 gene was localised to chromosome 17. Barker *et al* (1987b) showed linkage to two pericentromeric probes, D17S71 and D17Z1, and Seizinger and colleagues (1987c) to the nerve growth factor receptor (NGFR) in a study which included 5 of the Welsh families. NGFR was excluded as a possible candidate gene by the finding of recombinants, the maximum lod score being 4.41 at $\theta = 0.14$.

The formation of the International Consortium was an important step in 'speeding up' the mapping of the NF-1 gene. The exclusion map highlighted the areas of the genome on which efforts should be concentrated, thereby avoiding repetition of negative results using markers already studied. The spirit of collaboration established continued after the chromosomal localisation had been established.

The rapid pooling of data and materials by Consortium members resulted in the rapid progress towards the cloning of the NF-1 gene described in the final chapter.

1. Introduction

The mapping of NF-1 to chromosome 17 by linkage studies in May 1987 represented the first crucial step in applying the reverse genetics approach to the isolation and characterisation of the gene itself. The next steps in this approach are the exclusion of non-allelic heterogeneity by testing large numbers of families with the linked probes, and the accurate sub-chromosomal localisation of the gene using a number of approaches, which include identifying closely linked, flanking markers and physical mapping studies. The work involved in these strategies is enormous and can rarely be achieved by a single research group working in isolation. Following the mapping of the gene, the members of the International Consortium for NF-1 linkage continued to collaborate with regular pooling of data and resources, such as new probes and cell lines. This was made possible by the sponsorship of a series of workshops by the National Neurofibromatosis Foundation and enhanced by the rapid publication of the data presented at these meetings (*Genomics* 1:337-383, 1987; *American Journal of Human Genetics* 44:1-72, 1989). This degree of collaboration has resulted in extremely rapid progress towards the isolation of the gene itself.

In this chapter the progress made by January 1989 is described. The author's direct involvement in the work was as clinical co-ordinator for the laboratory based work in Cardiff.

2. Linkage analysis of chromosome 17 markers

A. Studies in the Cardiff family panel - The family panel for the chromosome 17 linkage studies comprised 22 families, 14 identified by the author through the South Wales population study and LINK (described in chapters 8 and 9), and a further 7 families ascertained through genetic counselling clinics in other parts of Wales (specimens collected by the author and Dr Alan Fryer) and one large family seen for counselling at the Kennedy Galton Centre, Harrow, by the author. This expanded family panel comprised 148 potentially informative meioses, 64 of which were phase known.

From the initial reports of Barker *et al* (1987b) and Seizinger *et al* (1987c), NF-1 appeared to be in the pericentromeric region of chromosome 17. The RFLPs identified by 16 DNA probes spanning from the proximal short to distal long arm of chromosome 17 were chosen for study in order to further define the sub-chromosomal localisation of the NF-1 gene. The laboratory analysis was performed by Dr M Upadhyaya and Ms W Broadhead; the statistical analyses were performed by Dr M Sarfarazi. The markers studied, their known physical localisation (at the outset of the studies) and the results of two point linkage analysis are shown in Table 10-1; the researchers who kindly made the probes available for these studies are also shown.

The results showed that eight pericentromeric markers (HHH202, EW206, CRI-L946, CRI-L581, EW203, EW301, p17H8 and FG2) were closely linked ($\theta \leq 0.10$) to NF-1. Loose linkage ($\theta > 0.10$) was observed with a further three markers (EW207, pA10.41, pe51) and four markers (EW205, heA1, EW204 and BS3) were not sufficiently informative within the families. No significant linkage was observed with hGH. Data on the

Table 10-1 Outcome of linkage analysis of 16 chromosome 17 DNA markers
and the Cardiff NF-1 family panel

The probes were kindly donated by: (a) Dr Y Nakamura; (b) Dr D Barker; (c) Dr K Stephens; (d) Dr H Willard; (e) Dr B Sykes; (f) Dr M Chao; (g) Dr G Moore; (h) Dr F Ruddle; (i) Dr P Seeburg.

Probe (source)	Locus	Physical localisation	\hat{z}	$\hat{\theta}$	Confidence interval
HHH202(a)	D17S33	17q	9.68	0.02	0-0.10
EW206(b)	D17S57	17q	4.16	0.04	0-0.18
CRI-L946(c)	D17S36	17	9.46	0.06	0.02-0.15
CRI-L581(c)	D17S37	17	6.06	0.06	0.02-0.17
EW203(b)	D17S54	17q	2.56	0.06	0.00-0.28
EW301(b)	D17S58	17p	11.79	0.08	0.03-0.16
p17H8(d)	D17Z1	17cen	4.53	0.08	0.00-0.22
FG2(e)	COLIA1	17q21.3-q22	3.38	0.10	0.03-0.26
EW207(b)	D17S73	17q	6.39	0.13	0.06-0.24
pA10-41(b)	D17S71	17p11	3.58	0.16	0.07-0.29
pE51(f)	NGFR	17q22	2.21	0.24	0.14-0.39
EW205(b)	D17S56	17q	0.27	0	0.00-0.11
hE A1(g)	ERBA1	17q11-q21	2.16	0.12	0.00-0.34
EW204(b)	D17S55	17q	0.16	0.17	0.08-0.35
B53(h)	HOX2	17q21-22	0.19	0.33	--
hGH(i)	GH	17q22-24	NO LINKAGE		

analysis of recombination frequency in males and females suggested a slight increase in recombination in females, but the numbers of meioses for each sex was too limited to draw statistically significant conclusions. Of the probes used, 5 identified a known gene locus; as NGFR had already been excluded as a candidate gene (Seizinger *et al* 1987c), only ERBA1 remained as a possible candidate for the NF-1 locus; the finding of 2/9 phase unknown recombinants excluded this possibility.

The data were analysed for heterogeneity using the program HOMOG (Ott, 1983), which assumes that there are two family types, one linked and the other unlinked. No evidence of heterogeneity was found.

The data were further analysed to see if the position of the NF-1 locus with respect to the centromere could be established. Several families contained multiply informative meioses from which the order of the probes could be inferred. The most informative was NF-17, which is shown with the genotypes of 5 of the closest linked markers in Figure 10-1. The pedigree provided information on the order of the NF-1 gene in relation to EW301, p17H8, HHH202 and EW206 (EW207 is uninformative). Individual IV-1 is a recombinant for EW301, p17H8 and HHH202 but not EW206. On the basis of this family, the likely order is pter-EW301-cen-HHH202-NF1-EW206-qter or pter-EW301-cen-HHH202-EW206-NF1-qter. As shown in Table 10-1, NF-1 is linked to HHH202 at a distance of 2cM and to marker EW206 at 4cM. Linkage data for the marker loci showed HHH202 to be linked to EW206 at $\theta=0.19$, $z=8.07$. If NF-1 were assigned to a location distal to EW206, the expected genetic distance between NF-1 and HHH202 would be $>19\text{cM}$, which is not compatible with the data. Therefore, based on this family, the likely order is EW301-cen-HHH202-NF1-EW206. This order was supported by the

NF17

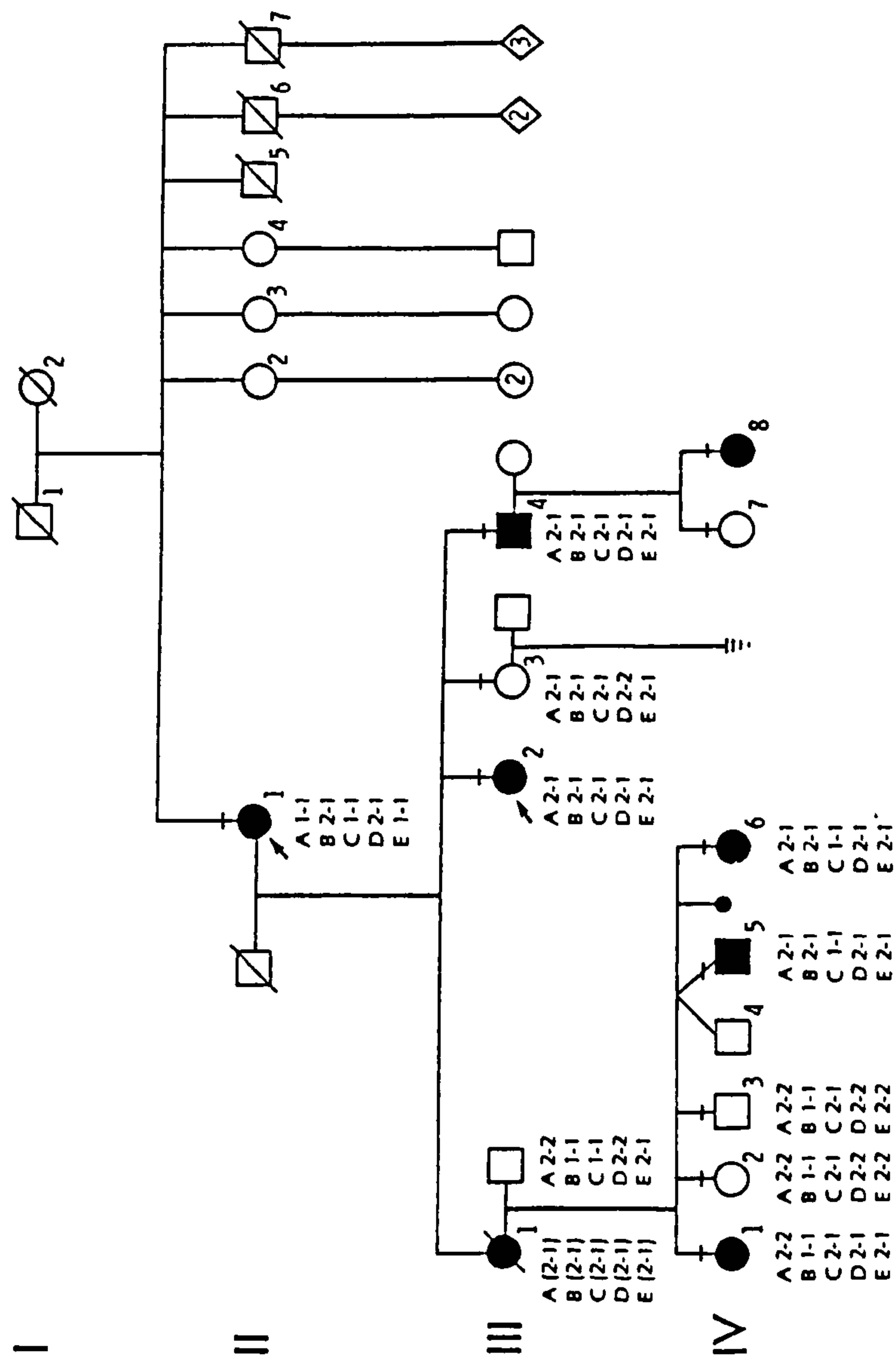


FIGURE 10-1. Family NF 17 typed with DNA probes EW 301 (A), p17H8 (B), HHH 202 (C), EW 206 (D) and EW 207 (E). Individual IV-1 is a recombinant for markers A, B and C but not D. The likely order of probes based on this family is pter-EW301-cen-HHH202-NF1-EW206-qter.

study of the other multiply informative meioses.

The programmes LINK and LINKMAP (Lathrop *et al* 1985) were used for multipoint analysis which placed NF-1 between HHH202 and EW206, with the likelihood of 83:1. The most likely order of the most closely linked probes on the basis of multiply informative meioses and multipoint mapping was pter-pA10.41-EW301-cen-HHH202-NF1-EW206-EW207-qter. This work therefore identified flanking markers for the NF-1 gene and placed the locus on the proximal long arm of chromosome 17. This work is presented in the paper of Upadhyaya and colleagues (1989).

B. The combined NF-1 linkage consortium data set - The purpose of linkage studies of markers around a disease locus is to establish the order of the markers and to establish accurate recombination fractions. This is essential for strategies towards cloning the gene itself and for clinical application. For these purposes, the more data available for analysis the more accurate the information obtained. Therefore, in June 1988 the NF-1 linkage Consortium agreed that the pooling of available data on the closely linked markers would be of great benefit. The outcome of this analysis is presented in the paper of Goldgar *et al* (1989) and is briefly reviewed below.

The combined data set included 142 families studied by eight groups and no suggestion of non-allelic heterogeneity was found. The data were analysed using the program CRI-MAP (Barker *et al* 1987c). The closest markers were HHH202 ($z=29.1$, $\theta=0.01$, upper 99% confidence limit 4%) and EW206 ($z=19.2$, $\theta=0.03$, upper 99% confidence limit 9%). The best order of the markers studied is shown, alongside the physical map of chromosome 17, in Figure 10-2; the distances given are

sex-averaged. Ten of the 13 loci were uniquely ordered with odds of $\geq 1000:1$. The 3 loci which could not be uniquely ordered at odds $\geq 1000:1$ (HHH202, EW204, HOX2) are placed in their most likely position; for mapping and clinical application, the most important of these is HHH202 which was placed with odds of 275:1.

For clinical application it is also important to know whether there is a significant difference in recombination between the sexes. Although overall this was the case, the total genetic distance from pA10.41 to NGFR being 26cM in males and 56cM in females, the closely linked flanking markers are still close enough to give a prediction with >95% accuracy in females as well as males (HHH202, $\theta_m=0.00$, $\theta_F=0.01$; EW206 $\theta_M=0.02$, $\theta_F=0.04$).

In summary therefore, linkage studies have shown no evidence of locus heterogeneity for NF-1 and identified closely linked flanking markers suitable for clinical application in prenatal/pre-symptomatic testing.

3. Physical mapping studies of the NF-1 region on chromosome 17

A. Cytogenetic studies in NF-1 patients - Prior to the mapping of the gene to chromosome 17 by linkage studies, routine cytogenetic investigations of NF-1 patients had not revealed any consistent abnormalities (Riccardi & Eichner 1986). Once the chromosomal localisation of the gene was known, cytogenetic analysis of patients with NF-1 could now focus on chromosome 17 in detail to see if patients, particularly new mutations or those with severe retardation, malignancy, or who were dysmorphic, had a visible cytogenetic rearrangement of the pericentromeric region.

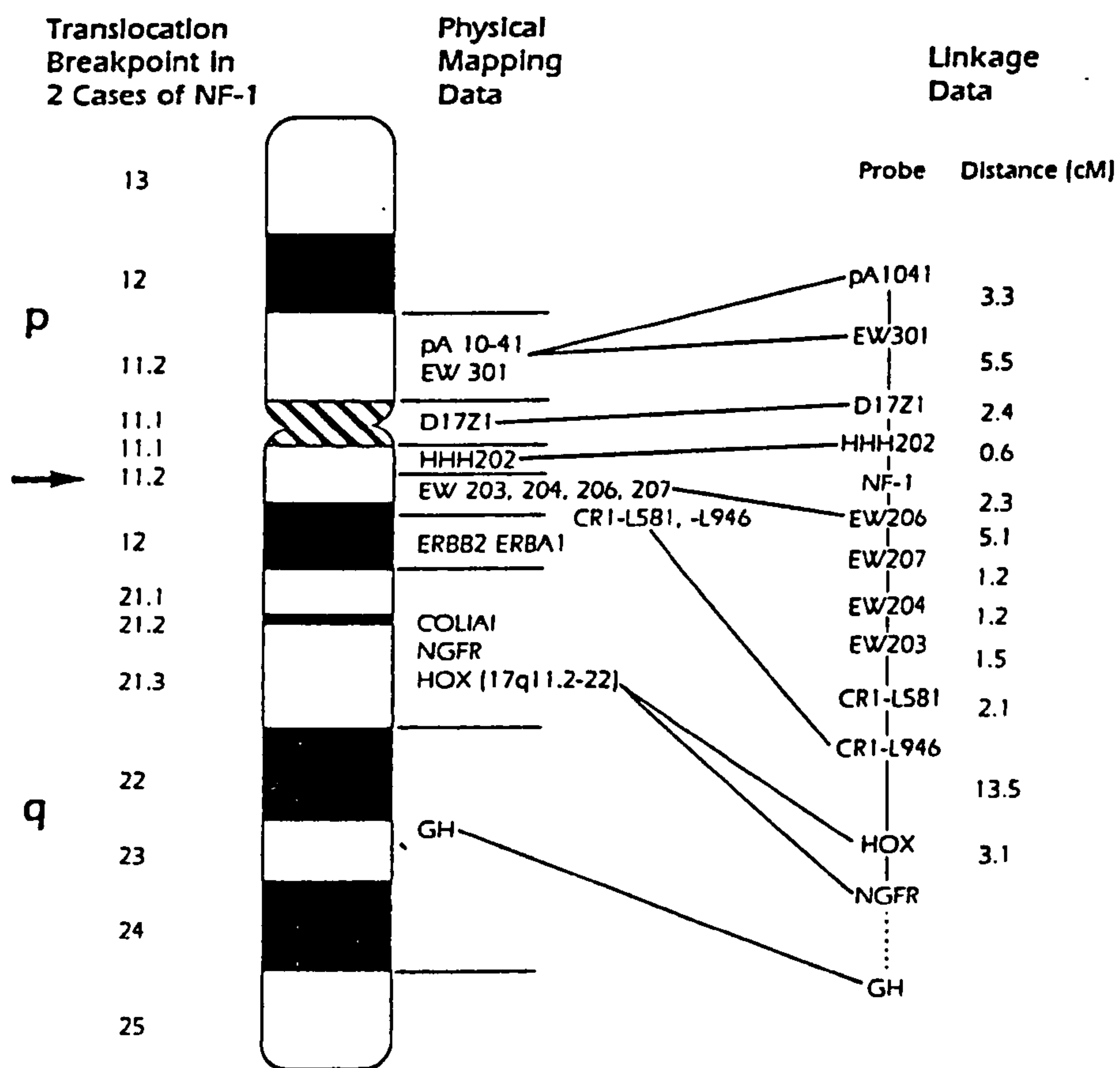


FIGURE 10-2. Physical and genetic map of chromosome 17 based on data available up to January 1989.

Nine of the patients in the population study have had normal cytogenetic investigation to date (28,53,68,79,82,108-111) and in a further 15 unrelated patients seen either for counselling at the Kennedy Galton Centre (by the author) or for assessment for a study of intellectual handicap in NF-1 (by Dr R Ferner) also had no visible cytogenetic abnormality.

Two cases have however been reported in the literature with balanced reciprocal translocations in NF-1 patients, which had a common breakpoint at 17q11.2. In the first case (Schmidt *et al* 1987), a mother (a probable new mutation for NF-1) and her two children with NF-1 were shown to have a t(1;17) (p34.3;q11.2) translocation. The chromosome analysis was initially undertaken in the mother because of a history of recurrent miscarriages in her sibship. In the second case (Ledbetter *et al* 1989), a new mutation for NF-1 was ascertained when her child, who had multiple congenital abnormalities and died at 10 months of age, was found to have an apparently balanced translocation involving chromosomes 17 and 22. The child is not reported to have had any NF-1 features prior to death. Detailed analysis of the mother's karyotype showed the breakpoints to be t(17;22)(q11.2;q11.2). Ledbetter *et al* (1989) have constructed a hybrid cell line from this mother which contains only the derivative chromosome 22, and mapping studies reported in the same publication and by Fountain *et al* (1989) showed that the markers that were shown to flank the NF-1 gene by linkage studies also flank the translocation breakpoint. It therefore seems likely that the translocation events in these two patients have disrupted the NF-1 gene and have identified its precise localisation.

B. Physical mapping studies using hybrid cell lines and Pulse Field Gel Electrophoresis (PFGE) - In Figure 10-2 the physical localisation of the probes used for the linkage studies in Section 1 are shown; these are based on the reports of Shaw *et al* 1987, Fain *et al* 1987, VanTuinen *et al* 1987 and Fountain *et al* 1989. The oncogene ERBB2 is included as it was another possible candidate gene; an RFLP at the ERBB2 locus has so far not been identified (Upadhyaya *et al* 1987) and therefore it could not be excluded as a candidate gene by linkage analysis. However, the physical mapping studies of Fountain *et al* (1989) have shown ERBB2 to be distal to the probable NF-1 locus and their PFGE analysis of NF-1 patient DNAs has failed to show rearrangements involving ERBB2. None of the markers studied so far has identified rearrangements in NF-1 patients on PFGE or identified the breakpoints in the two translocation cases (Fountain *et al* 1989).

C. Analysis of tumour DNA from NF-1 patients - With the mapping of the gene another immediate question which could begin to be addressed was the mechanism of tumour formation in NF-1. If the mechanism was analogous to that in NF-2 (see Chapter 1, Section 2F) then loss of heterozygosity would be seen in closely linked probes in tumour DNA when compared with lymphocyte DNA. Tumour material from patients seen by the author during the course of her work had been sent to Dr B Ponder to add to his extensive collection of NF-1 tumour material. Dr Ponder's initial work (personal communication, January 1989) has shown no loss of heterozygosity in simple neurofibromas; this is the experience of other centres (Dr B Seizinger, personal communication, January 1989). Dr Seizinger has however shown deletions on the short arm of 17 in 4 NF-1 neurofibrosarcomas, i.e. not involving the NF-1

gene, and therefore the trigger for tumour formation may involve other loci. In Dr Ponder's material 2 NF-1 phaeochromocytomas have shown loss of alleles with probes HHH202 and EW204, i.e. in the region of the NF-1 locus.

In summary, therefore, the mechanism of tumour formation in NF-1 is more complicated than can be explained by the hypothesis that the NF-1 gene is a tumour suppressor gene; of the findings to date only those from the phaeochromocytomas studied by Dr Ponder would fit this mechanism.

4. Conclusions

In summary, therefore, by January 1989, major locus heterogeneity for NF-1 had been excluded; the findings of physical and linkage studies had complemented each other in identifying closely linked flanking markers and the accurate sub-chromosomal localisation of the NF-1 gene had been identified. The cloning of the breakpoints in the two translocation cases, and therefore by implication the NF-1 gene, should be achieved in the near future.

The immediate clinical implications of the data presented in this chapter is that very accurate prenatal/presymptomatic diagnosis using closely linked flanking markers is now possible in families with a suitable structure. To this end, the guidelines for genetic counselling developed from the population study of NF-1 in South East Wales will be helpful in discussing these options with NF-1 families. However, as prenatal tests using linked probes will not predict disease severity and will not be applicable for the significant proportion of affected individuals who are new mutations, the uptake

by couples of these tests will probably be limited. The real hope for future care of NF-1 sufferers lies in the cloning of the gene, the identification of its product, eventual understanding of disease pathogenesis and development of possible treatments.

".... the reverse genetics trail that is currently being blazed for NF-1 shows a greater promise of providing an understanding of the true basis of this major genetic disease than has any previous endeavour in the more than a century since the original description of the disease."

Collins *et al* (1989)

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A P P E N D I C E S

APPENDIX A

Clinical Summaries of families seen.

Appendix A is divided into three sections:

Section 1 - in which the clinical details of families with NF-1 are presented; the family number is prefixed with NF.

Section 2 - in which the clinical details of index cases who had CAL spots as their only feature are presented; the family number is prefixed with DNF.

Section 3 - in which the clinical details of index cases and their families who had other forms of neurofibromatosis or diseases closely related are presented; the family number is prefixed with ONF.

Section 4 - the clinical details of index cases with a diagnosis other than NF-1 are presented; their number is prefixed with NNF.

Section 1

The families have been grouped according to the place of residence of the index case(s) as follows:

- (a) NF 1-40 resident in South Glamorgan;
- (b) NF 41-69 resident in West Gwent;
- (c) NF 70-73 index cases resident outside study area and ascertained for linkage studies.

For each family the index case(s) is described first, followed by details of relevant family members, beginning with generation I. For each affected individual the following details are listed:

- (a) date of birth
- (b) age when assessed for study
- (c) occupation*
- (d) social class*
- (e) details of education*
- (f) academic qualifications*
- (g) numbers of CAL spots >1.5 cm in diameter and <0.5 cm if ≤14 years old
- (h) areas of freckling
- (i) number of cutaneous neurofibromas (CNF) graded: none=1, few (<10)=2, scanty (10-99)=3, moderate (100-500)=4, extensive (>500)=5
- (j) facial appearance: normal=no, abnormal=yes, i.e. whether the diagnosis of NF-1 was obvious from the face, e.g. face:no means there were no obvious facial features of the disease.
- (k) Lisch nodules (LN): average no./eye, e.g. LN: 14.
- (l) grade of severity of NF-1 on a scale 1-4 (described in Chapter 2), e.g. GNF 1*
- (m) height in cm (centile for age), e.g. height: 90(>97) means the individual was 90 cm tall which was >97th centile.
- (n) head circumference in cm (centile for age), e.g. HC: 40 (50) means the head circumference was 40 cm and on the 50th centile.

*Denotes items of information listed only if individual resident in study area.

A brief clinical summary is then given if appropriate, with particular reference to NF-1 complications. The pedigrees of families NF1-73 follow the text of Section 1. For relatives found to be unaffected, the findings on cutaneous and slit lamp examination are given and other information (e.g. education, height) if this has been used in analysis. For all individuals, if a given piece of information is not stated, it was not available or examination was inadequate for the purpose.

In Tables A-1 and A-2 the study numbers of affected individuals used in the main text are cross-referenced with the pedigree numbers. In the case of affected individuals resident in South East Wales, the mode of inheritance and complications are also listed.

Table A-1 Study numbers of all affected individuals resident in
South East Wales cross-referenced with pedigree numbers,
mode of inheritance and complications

<u>Study No.</u>	<u>Pedigree No.</u>	<u>Mode of inheritance</u>	<u>Complication</u>
1	1,II-3	NM	-
2	1,III-3	M	1,2
3	1,III-4	M	2
4	1,III-5	M	2,9
5	2,II-1	NM	8
6	3,II-2	NM	8
7	3,III-1	F	8
8	3,III-2	F	-
9	3,III-3	F	8
10	4,II-1	NM	3
11	5,II-9	NM	1
12	6,III-2	NM	1,2
13	7,III-3	M	8
14	7,III-6	M	-
15	7,III-7	M	-
16	7,III-8	M	-
17	7,III-9	M	9
18	7,IV-1	F	-
19	7,IV-4	F	1,2,13
20	7,IV-6	F	1,2,3
21	7,IV-7	F	3
22	7,IV-8	M	1
23	7,IV-10	M	-
24	8,III-3	F	3,5,7
25	9,II-1	NM	1,2,8
26	9,III-1	F	1,8
27	10,II-1	NM	-
28	11,II-1	NM	1,9
29	12,III-8	M	2
30	12,IV-7	F	-
31	12,IV-8	F	1,2
32	13,II-1	U	-
33	13,III-2	M	-
34	14,III-1	M	2,9
35	14,III-2	M	2
36	15,II-1	M	1,2,3
37	15,II-2	M	1,2
38	15,II-4	M	-
39	15,III-1	F	-
40	16,II-4	U	-
41	17,II-1	NM	13
42	17,II-2	M	13
43	18,III-1	F	-
44	19,II-3	NM	2
45	19,III-3	M	-
46	20,II-1	NM	-
47	20,III-1	M	-
48	20,IV-1	M	2,4
49	20,IV-2	M	1

50	21,IV-2	M	1
51	21,IV-3	M	-
52	22,II-1	U	-
53	23,II-2	NM	1,2
54	24,III-5	M	2
55	25,II-8	NM	-
56	25,III-2	M	-
57	26,II-1	NM	1,3
58	27,II-1	NM	-
59	28,II-2	NM	-
60	28,III-1	M	1
61	29,II-1	U	1,2,3,11
62	29,IV-2	M	1,2
63	29,IV-3	M	2
64	30,III-1	F	-
65	30,IV-1	M	3,6
66	30,IV-3	M	1,8
67	31,II-4	NM	12
68	32,II-2	NM	1,2
69	33,IV-4	M	8
70	34,II-3	NM	1
71	34,III-3	F	15
72	35,II-3	NM	-
73	36,III-1	F	-
74	37,II-16	NM	2
75	38,II-2	M	2
76	38,III-1	M	1,2,3
77	38,III-2	M	2,3
78	39,II-4	NM	-
79	39,III-2	F	1,2
80	40,II-3	F	-
81	41,III-1	M	1
82	41,IV-1	M	2,14
83	42,II-6	NM	1
84	42,III-3	M	1
85	42,III-4	M	2,14
86	42,III-6	M	-
87	43,II-7	NM	-
88	44,III-1	M	-
89	44,IV-1	M	1,3,4
90	45,II-3	NM	-
91	46,II-5	NM	-
92	46,III-3	M	-
93	47,II-9	M	-
94	48,II-5	NM	1,2,5,6,12
95	49,I-1	U	-
96	49,II-1	M	2
97	49,II-2	M	2
98	49,II-3	M	-
99	49,II-7	M	-
100	49,III-1	M	2
101	49,III-2	M	2
102	49,III-3	M	-
103	49,III-4	F	-
104	49,III-5	F	-
105	49,III-7	M	2
106	49,III-8	M	-
107	49,III-9	M	-
108	50,II-1	NM	1,2,8

109	51,II-2	NM	1
110	52,II-2	NM	-
111	53,III-2	F	9
112	54, N.P.*	U	1,2
113	55,II-3	NM	1
114	56,II-1	M	-
115	56,III-3	M	1
116	56,IV-3	M	1
117	57,II-1	F	-
118	58,II-5	NM	2,11
119	59,II-2	NM	2,10
120	60,II-1	NM	8
121	60,III-2	M	-
122	61,II-2	NM	1
123	62,III-3	F	1,2
124	63,II-2	NM	-
125	63,III-5	F	-
126	64,II-4	NM	-
127	64,III-1	M	-
128	64,III-2	M	1
129	65, N.P.	NM	8
130	66,II-2	NM	2
131	67,II-1	NM	1,2
132	67,III-1	F	1
133	68,III-1	F	8
134	68,IV-2	M	-
135	69, N.P.	F	-

Key to Table A-1:

- (1) N.P.* = no pedigree
- (2) For mode of inheritance - M = mother affected; F = father affected;
NM = new mutation; U = uncertain.
- (3) The complications of NF-1 are coded as follows:
1 = plexiform neurofibromas; 2 = intellectual handicap; 3 = epilepsy;
4 = glioma; 5 = spinal neurofibroma; 6 = aqueduct stenosis;
7 = meningioangiomas; 8 = scoliosis; 9 = pseudoarthrosis;
10 = lambdoidal suture defect; 11 = endocrine tumours; 12 = renal
artery stenosis; 13 = visceral neurofibromas; 14 = delayed puberty
and 15 = congenital glaucoma.

Table A-2 Study numbers, cross-referenced with pedigree numbers, for other affected family members specifically mentioned in main text of thesis.

<u>Study Number</u>	<u>Pedigree Number</u>
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Deceased affected relatives

137	12,II-6
138	24,II-4
139	7,IV-9
140	53,IV-3
141	29,III-3
143	17,III-1
144	44,II-1
145	15,I-1
146	7,II-1
147	68,II-1
148	21,III-1
149	33,III-2
150	38,I-2
151	12,II-3
152	13,III-1
153	1,I-1
154	8,II-3
155	33,II-1
156	7,III-5
157	18,II-1
158	22,II-5
159	30,II-2
160	41,II-8
161	62,II-1
162	36,II-4
163	24,II-1

Affected relatives with CNS tumours or malignancy

136	47,III-1
142	36,III-2

Other affected relatives specifically mentioned

164	24,IV-1
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NF 1

II-3 was ascertained both by referral from her general practitioner (GP) and medical records.

II-3 - 15.1.28, 57.3 years.

Housewife; SC: V; attended secondary modern school, no qualifications.

8 CAL; freckling: axillae and submammary region; CNF: 4; face: no; LN: 20; GNF: 2; Ht: 150(<3); HC: 57.5(>97).

II-3 had had 2 cutaneous neurofibromas removed in 1969 and thought NF-1 'was just a skin problem'. She did not understand the genetic implications of NF-1 until seen for the study. On examination the only other problem was long-standing weakness of the evertors and dorsiflexors of the right ankle; this was the result of diphtheria at the age of 9 years.

I-1 - born 1885, died aged 57 years, 1942.

II-3 reported multiple 'lumps' like her own in her father but knew of no details of his parents or siblings. He also had rheumatoid arthritis and died from ischaemic heart disease (case no. 153 in cause of death analysis).

II-1 - born 1924. Lived outside study area, reported normal.

II-2 - born 1926. Lived outside study area, reported normal.

III-1 - 9.5.47, 38 years.

Housewife, SC: V; attended comprehensive school, no qualifications. Examination of skin normal. Height 162.5 (50). Both children reported normal.

III-2 - 28.1.49, 36.3 years.

Distribution assistant (Gas Board), SC: IV; attended secondary modern school, no qualifications. Examination of skin normal. Ht:170 (25). Daughter reported normal.

III-3 - 28.5.51, 33.9 years; coalman (unemployed); SC: V; remedial class education; no qualifications.

10 CAL; freckling: axillae; CNF: 3; face: no; LN: 50; GNF: 3; Ht 168 (10); HC: 60 (>97).

III-3 was entirely asymptomatic and was unaware that he had NF-1. He had a small plexiform neurofibroma on his left lower back with overlying skin hypertrophy and hypertrichosis.

III-4 - 21.6.56, 28.9 years; coalman (unemployed). SC: V; special school education, literate.

7 CAL; freckling: axillae and groins; CNF: 3; face: no; LN: not examined; GNF: 3; Ht: 173 (50); HC: 57 (90).

Entirely asymptomatic, unaware of diagnosis and had never had a hospital assessment.

III-5 - 6.9.57, 27.7 years; coalman; SC: V; special school education, literate.

12 CAL; freckling: axillae and groins; CNF: 3; face: no; LN: not examined; GNF: 3; Ht: not appropriate; HC: 57.5 (97).

III-5 had been noted to have an abnormal left foot when he started walking (at 14 months). Orthopaedic referral showed a valgus deformity secondary to pseudoarthrosis of the fibula. Radiology showed irregularity of the distal tibial epiphysis, with retonation of growth on the left side and complete absence of a 2 cm segment of the fibula just proximal to the epiphyseal line. He had 3 corrective orthopaedic operations during childhood (1960, 1968 and 1969). Resulted in fusion of fibula with good mobility, although ankle still abnormally shaped on examination. No mention of NF-1 features in his orthopaedic notes, nor of his mother being affected. He had had no other problems.

III-6 - 21.7.63, 21.8 years. Housewife; SC: V; attended comprehensive school, no qualifications. Examination of skin normal. Ht: 153 (10). Both children reported normal.

II-1 was referred by both her GP and neurologist.

II-1 - 3.7.40, 44.6 years. Secretary (personal assistant); SC: II; educated at private high school, attained GCE 'O' levels.

6 CAL; freckling: none; CNF: 4; face: no; LN: 10; GNF: 2; Ht: not appropriate; HC: 56.5 (>97).

II-1 was diagnosed at 20 years when she presented with a peptic ulcer. During treatment (with bed rest) she complained of backpain, also slight frequency and urgency of micturition. Examination of the lumbar spine showed a scoliosis at L1-3. Plain radiographs showed marked scalloping of the lumbar vertebrae but myelography showed only dural ectasia and no other lesion. No active treatment was undertaken.

Apart from intermittent slight back pain, she was then well until 1984 when she developed pain in the distribution of L1 which was aggravated by coughing. Examination showed no focal neurological signs. Repeat myelography (Figure 5-4) and CT of the spine showed increase in the degree of scalloping and scoliosis; the nerve roots were displaced by the dural sac. The pain was presumed to be related to the dural ectasia but as the myelogram had excluded a space occupying lesion, no active treatment was advised.

Her only other NF-1 problem was a neurofibroma in the gum of the upper jaw. She was under periodic review for this. She was unaware of the genetic implications of NF-1 when seen for study.

Classified as new mutation.

I-1 - 30.8.05, died 1973.

His wife was sure that I-1 had no NF-1 skin lesions. Died of an abdominal reticulosarcoma. Autopsy report reviewed: skin normal.

I-2 - 1.11.05, 80.2 years

1 CAL on examination, slit lamp examination normal, i.e. no evidence of NF-1.

III-1 - 10.3.65, 20 years. Waitress; SC: IV; attended comprehensive school, attained GCE 'A' level passes.

2 CAL on examination; slit lamp examination normal.

III-2 - 7.1.67, 18.1 years. Bank clerk; SC: III(N); attended comprehensive school, attained GCE 'O' level passes. Examination of skin and iris normal.

NF 3

Ascertained via III-1, who was referred to the study by a consultant clinical geneticist and her GP independently; her records were also ascertained via the Prince of Wales Orthopaedic Hospital.

III-1 - 31.10.55, 28.9 years. Housewife; SC: IVM; attended comprehensive school, no qualifications.

4 CAL; freckling: no; CNF: 3; face: no; LN: 3; GNF: 3; Ht: not appropriate; HC: 55.5 (90).

Developed left thoracolumbar scoliosis at 9 years and referred for orthopaedic assessment. Curve involved T10-L2, 33°, vertebrae normal. Progression of curve over one year to 68°, posterior final fusion performed in January 1967. Successful correction to 25° but relapsed in 1968 to 95°, further fusion undertaken in 1969, final correction to 70°. Left with marked residual kyphoscoliosis but no pain and therefore no further operations were undertaken until she presented with severe pain over her curve in 1985. Re-exploration showed a stable fusion with 2 nerve roots trapped. These were released with resolution of symptoms.

III-1 had no other medical problems but she was unaware her spinal condition was related to NF-1.

I-1 - 15.11.1880, died 77 years.

I-1 died as a result of a stroke.

I-2 - 2.10.1898, died 73 years.

Died from left ventricular failure secondary to a myocardial infarction. II-2 was sure both his parents had no stigmata of the disease.

II-1 - 4.5.21. II-1 and offspring reported normal.

II-2 - 10.1.25, 59.7 years. Clerical officer; SC: IIIN, attended elementary school, no qualifications.

0 CAL; freckling: axillae; CNF: 4; face: no; LN: 2; GNF: 2; Ht: not appropriate; HC: 56 (75).

Slight curvature of the spine noted on naval entrance at 22 years. Asymptomatic, no progression, minimal left thoracic kyphoscoliosis on examination. Diagnosed as having NF-1 when his daughter developed severe scoliosis. Classified as new mutation.

II-3 - 27.10.26. He and one child reported normal.

II-4 - 12.11.30, reported normal.

II-5 - 28.12.31, 53.9 years. Fitters mate; SC: IV; attended elementary school, no qualifications.

Reviewed as family had noted CAL spot on chin of III-4. Cutaneous examination of II-5 normal. III-4 had a 1 cm diameter CAL mark on her chin but nothing else. Both her and her father classified as normal.

II-6 - 3.5.34, reported normal. First child had died in infancy, cause uncertain, remaining children said to have no problems.

III-2 - 23.5.58, 26.3 years. Painter and decorator (unemployed); SC: IIIM; attended comprehensive school, no qualifications.

5 CAL; freckling: no; CNF: 3; face: no; LN: 6; GNF: 1; Ht: 170 (25); HC: 57.5 (97).

III-3 - 16.3.60, 24.5 years. Factory inspector; SC: IVM; attended comprehensive school, no qualifications.

6 CAL; freckling: axillae; CNF: 3, face: no; LN: 20; GNF: 2; Ht: not appropriate; HC: 55.5 (90).

Had been reviewed by an orthopaedic surgeon in late teens for slight lumbar scoliosis. Notes untraceable. On examination, asymptomatic slight left lumbar scoliosis, no investigations undertaken.

II-1 was referred to the study by his GP and also ascertained via medical records.

II-1 - 26.6.32, 52.1 years. Received invalidity benefit, had formerly worked as clerical officer until made redundant in 1979 when could find no further employment; SC: IIIN; attended grammar school, no qualifications.

4 CAL; freckling: axillae; CNF: 5; face: yes; LN:6; GNF: 3; Ht: 174 (50); HC: 58 (97).

Developed epilepsy in his late teens; his fits took the form of complex partial seizures when he experienced depersonalisation, mumbled incoherently and clenched his right fist. NF-1 was diagnosed by a Consultant Neurologist in 1957 and he had been intermittently reviewed by the Department of Neurology since that time. He seems at first to have been reasonably controlled on a phenytoin/phenobarbitone mixture but in 1979 the frequency of attacks increased and he also developed a gait disturbance; a spinal neurofibroma was queried. EEG showed generalised abnormality and suggestion of left temporal focus; myelogram was normal. The frequency of his fits decreased spontaneously with no change in medication.

At the time of review for study, he showed mild inco-ordination of the limbs with gait ataxia, reduced knee and absent ankle jerks, his left plantar was extensor. Similar findings had been noted in 1977. Full neurological review was undertaken (by Dr Compston) and CT scan showed the left lateral ventricle was enlarged compared with the right and a degree of cerebral atrophy. It was felt that his cerebellar disturbance and peripheral neuropathy were complications of long-standing phenytoin treatment and an underlying cause of his seizures was not identified. As he was extremely anxious regarding his attacks and his disease generally, it was felt that at least some of his attacks may be due to panic.

Classified as a new mutation. He and his wife had tried to have children for a number of years, but with no success. No formal investigations for infertility had been undertaken.

I-1 - born 1895, died 68 years from carcinoma of the lung.

I-2 - born 1900, died 76 years from carcinoma of the pancreas.

II-1 was sure both his parents were unaffected. There was no history of NF-1 in their extended families.

NF 5

II-9 was referred by her GP and also ascertained by the dermatology/histology index.

II-9 - 25.7.06, 78 years. Retired former housemaid, SC: IV; attended normal school, no qualifications.

2 CAL; freckling: none; CNF: 5; face: yes; LN:12; GNF: 3; Ht: 156 (10); HC: 54 (50).

II-9 was aware of being different from her siblings from the time she began to develop cutaneous neurofibromas. Her only medical treatment in relation to NF-1 through the years had been to have the largest of her cutaneous neurofibromas removed. Her only other problem was bilateral deafness, from the age of 27 years, the cause of which was uncertain; she coped well with the use of a hearing aid. Her mother, II-1 and II-5, had had similar hearing problems.

On examination she had bilateral conductive deafness and no other neurological signs. She had a small plexiform neurofibroma on her left arm with overlying skin hypertrophy and pigmentation.

II-9 felt NF-1 had "ruined her life" and she had never been swimming or gone out without her arms covered. Why she had not had children was not discussed because it was felt she was already distressed by the interview.

Classified as a new mutation.

I-1 - born 1862. Died 81 years from uraemia secondary to complications of a prostatectomy operation.

I-2 - year of birth uncertain. Died 76 years, cause uncertain.

The propositus was adamant she was the only affected family member.

Details regarding her siblings were vague other than this fact. Six of her siblings were deceased and the details were as follows:

- II-1 - died 78 years from Parkinson's disease;
- II-2 - died 86 years from carcinoma of the oesophagus;
- II-3 - died 64 years, cause unknown;
- II-4 - age and cause of death unknown;
- II-6 - died 80 years from cancer, site unknown;
- II-10 - died 76 years as a result of a stroke;

The remainder of her siblings were healthy.

NF 6

III-2 was referred both by a consultant dermatologist and her GP.

III-2 - 23.2.65, 19.5 years. Packer in shoe factory; SC: IVM; remedial class education, obtained some CSE passes with relatively low grades (4 and 5).

14 CAL; freckling: axillae; CNF: 4; face: no; LN: 51; GNF: 3; Ht: 162.5 (50); HC: 57 (>97)

NF-1 had been diagnosed at 10 years when her parents became concerned about her appearance. No details regarding the nature of her disease had been discussed. She had had one cutaneous neurofibroma removed prior to being seen because of pressure from a strap.

Although her motor milestones had been normal, her speech development had been delayed. She had required remedial class education and still experienced difficulty with reading. Psychometric examination was undertaken after she had been seen for the study (by Dr Vingoe, Principal Psychologist, UHW), this showed verbal IQ 90, performance IQ 101 and full-scale IQ 94. She was therefore of average intelligence but it was noted that her attention span was particularly poor; it was felt her performance was marred to some extent by very low self-esteem.

On examination, she had severe NF-1 manifestations for her age and a 10x10 cm plexiform neurofibroma over the right iliac crest. Classified as a new mutation.

I-2 - 14.8.10, 74 years.

The parents of III-2 had asked her to attend when the family were assessed because she had one CAL spot and numerous freckles. The CAL spot was confirmed on examination but the freckles were only in exposed areas of the body. There was no history of NF-1 in her or her husband's extended families.

II-1 - 22.12.41, 42.6 years. Detective Sergeant of police. SC: IIIN; no abnormality of skin or iris on examination. Ht: 183 (90). No history of NF-1 in either of his parent's extended families.

II-2 - 14.1.41, 43.6 years. State Enrolled Nurse; SC: II. No abnormality of skin or iris on examination; Ht: 165.5 (75).

III-1 - 2.3.64. Reported normal.

III-3 - 29.1.67 Reported normal

III-4 - 25.3.71, 13.4 years.

On examination, 1 CAL spot; normal irides.

NF 7

Three affected individuals in this large family were independently ascertained: III-3 (via his GP, orthopaedic and genetic records), III-9 (referred by her GP) and IV-4 (general hospital records and GP).

III-3 - 18.11.41, 42.5 years. Sewerman; SC: IV; secondary modern school education, no qualifications.

4 CAL; freckling: axillae; CNF: 4; face: yes; LN: 0; GNF: 3; Ht: not appropriate; HC: 54 (25)

Presented with right thoracolumbar scoliosis age 9 years (1958); 90° curve from T10-L3, vertebrae normal. Posterior spinal fusion performed September 1959, curve improved to 70°. In 1969, pain over the residual kyphoscoliosis led to re-exploration, no problem detected. Left with relatively severe residual deformity and still

experienced intermittent pain at time of study assessment.

He had been aware of the diagnosis of NF-1 since childhood. Apart from not swimming because of his appearance, he had no other related problems. On examination on two occasions for the study his blood pressure was marginally elevated to 150/100 erect and supine; 24 hour urinary VMA was normal on one occasion and he declined further investigations.

III-9 - 4.1.54, 30.5 years. Housewife; SC: IIIM; secondary modern school education, no qualifications.

10 CAL; freckling: axillae and over whole trunk; CNF: 3; face: no; LN: 23; GNF: 3; Ht: not appropriate; HC: 53 (10)

Her mother had noted bowing of the right tibia on the third day of life; orthopaedic referral showed severe bowing of the right tibia in association with multiple CAL spots and the diagnosis of NF-1 was made. Wore a caliper until 9 years, but shortly after its removal, III-9 fractured her tibia and non-union resulted. Had various orthopaedic procedures with no success, below knee amputation performed in 1968.

Although the orthopaedic notes clearly state the diagnosis of NF-1, III-9 thought her leg problem was secondary to osteomyelitis. She had no other problems until just prior to her assessment when she had developed intermittent severe thoracic pain and was referred for neurological assessment. There were no localising signs but a myelogram was performed to exclude an intraspinal lesion, this was normal. Following this a subcutaneous neurofibroma in the region of the pain was removed with partial resolution of symptoms.

IV-4 - 21.12.75, 8.6 years. Schoolgirl; SC: IIIM; remedial class education.

8 CAL >1.5 cm, 0>0.5 cm; freckling: axillae; CNF: 1; face: no; LN: 2; GNF: 3; Ht: 117 (3); HC: 50 (25).

IV-4 had presented at the age of 6.5 years with anorexia and general malaise. Examination had shown multiple CAL spots and a mass in the right ileac fossa. At laparotomy a single large benign neurofibroma was found in the mesentery of the right colon; hemicolectomy and terminal ileectomy was performed with resolution of the symptoms.

She attended a remedial class and a psychological assessment in

February 1982 showed (on Wechsler Intelligence Scale) verbal IQ 71, performance IQ 82, full-scale IQ 75. Assessed as being below average ability with marked learning difficulties.

On examination for the study, found to have 2 plexiform neurofibromas: an indiscrete mass above her left nipple with overlying skin hypertrophy and hypertrichosis and a similar lesion on the left thigh.

II-1 - 19.6.11, died 68 years.

The diagnosis of NF-1 had been made in 1955 when she presented with unrelated gynaecological problems. At that time she had multiple CAL spots and cutaneous neurofibromas (one of these was biopsied, histology of a benign neurofibroma). In October 1979 presented with anorexia and weight loss, carcinoma of the stomach was found at laparotomy. She died 2 days after a gastro-oesophagectomy with pulmonary oedema. No other abnormalities were detected at autopsy. Her family history was obtained from her spouse who reported that II-1 was the first case of NF-1 in her family.

III-1 - 8.1.38, 48 years.

Examination of skin normal.

III-2 - 25.10.39, 45 years.

Examination of skin and iris normal.

III-4 - Born 1942, died at 3 months.

Her father did not recall noting CAL spots prior to her death from septicaemia.

III-5 - 2.1.52, died 27 years.

Medical details obtained from wife. Had numerous CAL spots and neurofibromas at time of marriage, age 25 years, but had no other NF-1 related problems. Then had recurrent episodes of severe depression, was intermittently violent towards his wife; committed suicide by hanging in 1979.

III-6 - 30.1.48, 36.4 years. Sewerman; SC: IV, secondary modern school education, no qualifications.

14 CAL; freckling: axillae, all over trunk; CNF:5; face: yes; LN: 43; GNF: 2; Ht: 162 (3); HC: 55.5 (50-75).

III-6 was extremely anxious about his appearance and had had several of the larger cutaneous neurofibromas removed.

III-7 - 22.11.49, 35.2 years. Sewerman; SC: IV; secondary modern school education, no qualifications.

9 CAL; freckling: axillae and groins; CNF: 4, face: no; LN:18, GNF: 2; Ht: 158 (<3); HC: 55 (50).

No problems directly related to NF-1. Intermittent bouts of depression and excess alcohol intake for which he had had psychiatric review.

III-8 - 4.6.52, 32.1 years. Housewife; SC: IIIM; secondary modern school education, no qualifications.

9 CAL; freckling: axillae and over whole trunk; CNF: 4; face: no; LN: 27; GNF: 2; Ht: 148.5 (<3); HC: 54.5 (50)

Had had several cutaneous neurofibromas removed for cosmetic reasons. Unaware of genetic implications of NF-1 and uncertain whether children were affected until study assessment.

IV-1 - 10.9.70, 13.8 years. Schoolgirl; SC: IV; secondary modern school, no problems.

21 CAL >1.5 cm, 0 >0.5 cm; freckling: axillae and over whole trunk; CNF: 1; face: no; LN: 6; GNF: 1; Ht: 148 (3); HC: 59.5 (>97)

Correctable divergent squint of left eye secondary to myopia from early childhood. When assessed for LN study, optic discs normal and no further investigations undertaken.

IV-2 - 11.12.71, 12.6 years. Attending remedial class at comprehensive school.

Normal examination of skin and iris. Ht: 129.5 (<3)

Excluded from linkage analysis as father's siblings cast doubt on paternity of this branch of the family.

IV-3 - 9.1.73, 11.6 years. Attending comprehensive school, no problems.

Normal examination of skin and iris. Ht: 140 (25).

Excluded from linkage analysis because of question of paternity.

IV-5 - 19.4.78, 6.2 years. Attending remedial class at primary school.

Examination of skin showed 5 extremely pale CAL spots 1 cm in diameter. Slit lamp examination normal. Classified as equivocal, excluded from analysis.

IV-6 - 23.5.77, 7.1 years. Schoolgirl; SC: IV; remedial class education.

10 CAL >1.5 cm, 0 > 0.5 cm; freckling: unilateral axillary on left; CNF: 2; face: no; LN: 40; GNF: 3; Ht: 115 (25); HC: 51 (25)

Diagnosed secondary to study. Approximately 6 months after being assessed, developed absence attacks and EEG showed modified spike and wave activity. Good control achieved with sodium valproate therapy. On examination a small plexiform neurofibroma with overlying skin hypertrophy was noted below her right ear.

IV-7 - 6.3.82, 2.3 years. SC: IV, achieving normal milestones.

2 CAL >1.5 cm, 8 >0.5 cm; freckling: none; CNF: 1; face: no; LN: present, unable to count accurately; GNF: 3; Ht: 89(25); HC: 50(50).

Diagnosed secondary to study. Shortly after being seen, had 2 grand mal seizures and probably some absence attacks in addition. Sodium valproate therapy commenced and no further seizures; EEG normal.

IV-8 - 25.9.71, 12.8 years. Schoolboy; SC: IIIM; comprehensive school, no problems.

6 CAL >1.5 cm, 0 > 0.5 cm; freckling: none; CNF: 1; face: no; LN: 33; GNF: 2; Ht: 133 (<3); HC: 55 (75)

Diagnosed secondary to study. On examination noted to have large area of CAL pigmentation in bathing trunk distribution with a plexiform neurofibroma, several cm in diameter, in the middle (over the lumbar spine). This was intermittently painful and he was therefore referred to a plastic surgeon. Lumbar spine x-ray showed no significant abnormality. At operation the lesion was found to extend down into the buttocks therefore only the painful area was excised. Histology confirmed the clinical diagnosis and there was some resolution of pain.

IV-9 - 18.2.73, died 7 months.

No problems until August 1973 when developed severe diarrhoea. On hospital admission a suprapubic mass was noted and at subsequent laparotomy a tumour invading the bladder, uterus and pelvic wall was found. Histology showed an embryonal rhabdomyosarcoma. She died soon after the operation. Her mother remembered one CAL spot and the hospital notes commented on multiple lesions. Mother was unaware of the relationship of her tumour to NF-1.

IV-10 - 15.5.76, 8.1 years. Schoolboy; SC: III; attending primary school with no educational problems.

6 CAL >1.5 cm, 2 CAL >0.5 cm; freckling: none; CNF: 2; face: no; LN: 26; GNF: 1; Ht: 121.5 (25); HC: 55.1 (97).

Diagnosed secondary to study.

IV-11 - 25.1.78, 6.4 years. Attending primary school, no educational problems.

Examination showed 1 CAL spot but no other features (slit lamp examination performed); Ht: 108.5 (3).

Excluded from initial linkage study but re-examination one year later showed no other lesions, therefore classified as normal in subsequent studies.

IV-12 - 28.10.78, 5.7 years. No educational problems.

Normal examination of skin and iris. Ht: 112.5 (50).

IV-13 - 5.10.83. 0.7 years. Achieving normal milestones.

Normal examination of the skin.

NF 8

III-3 was referred to the study by his neurosurgeon and also ascertained via medical records.

III-3 - 9.12.30, 53.5 years. Former senior accounts clerk, made redundant in 1980, registered disabled when seen; SC: II; attended grammar school, achieved School Certificate and took an accounting

course at a College of Further Education.

16 CAL; freckling: none; CNF: 3; face: no; LN: not examined; GNF: 4; Ht: 166 (10); HC: 58 (97).

NF-1 had been diagnosed in 1961 when a painful neurofibroma had been removed from an intercostal nerve. In 1971 he presented with neck pain and difficulty in walking and was referred to a neurosurgeon.

On examination he had a spastic tetraparesis and loss of proprioception in the right hand. Myelography showed probable neurofibromas at C1 and C2. At operation a large neurofibroma was removed at C2 and a much smaller lesion at C1 was left. He had complete resolution of symptoms initially but his problems recurred in 1973 and the neurofibroma at C1 was removed. His only residual problem was loss of proprioception in the right hand.

He was well until 1974 when he presented with headaches and drowsiness. Investigation showed a right frontal tumour and subtotal removal was undertaken; histology was reported as showing a well-differentiated astrocytoma after which he was treated by radiotherapy. Three years later he developed a left hemiparesis, CT showed a right parietal lesion; after surgical decompression he was managed intermittently on dexamethasone for 7 years until his condition deteriorated, at which time CT demonstrated communicating hydrocephalus but despite insertion of a ventricular shunt, his condition had not improved by 1985. The frontal tumour was histologically reviewed at the time of shunt insertion and reclassified as meningioangiomatosis. He was seen twice during the course of the study and his left hemiparesis was slowly progressing, he had developed loss of inhibition and short-term memory.

The details of his family history were supplied by his sister, III-2, an associate specialist in genito-urinary medicine. She reported that both herself and III-1 had no features of NF-1 but that their father (II-3, date of birth 11.9.91) had been affected. He had had no complications of NF-1 but had had multiple CAL spots and cutaneous neurofibromas. He died at the age of 45 years from cardiac failures secondary to myocarditis. Neither his parents nor siblings were reported affected.

III-1 was referred to the study by her GP, she was also ascertained by the genetic and orthopaedic records.

III-1 - 6.1.60, 24.4 years. Clerical assistant; SC: IIIN; comprehensive school education, attained 6 'O' level and 2 'A' level passes.

14 CAL; freckling: axillae; CNF: 2; face: no; LN: 3; GNF: 3; Ht: not appropriate; HC: 55 (90).

NF-1 was diagnosed at 11 years of age when she presented with multiple CAL spots and right thoracic scoliosis (curve 55°, T5 - T10, vertebrae normal). This progressed over the next year to 66° and a posterior spinal fusion was performed. The curve was corrected to 30° and remained stable with good cosmetic effect. III-1 had been seen for genetic counselling at 17 years of age.

On examination the only other feature of note was a large plexiform neurofibroma on the right thigh (approximately 20 cm in diameter). The skin overlying it was hypertrophied in the centre and there was surrounding CAL pigmentation.

II-1 - 21.10.29, 54.6 years. Garage proprietor; SC: IIIM; secondary modern school education, no qualifications; reported difficulty in learning to read requiring extra help in this area alone.

15 CAL; freckling: axillae and groins; CNF: 4; face: yes; LN: 17; GNF: 2; Ht: not appropriate; HC: 59 (>97).

NF-1 was diagnosed when his daughter presented with scoliosis. In 1982 he had a neurofibroma removed from his right upper eyelid and had residual slight ptosis. He had been aware of a slight scoliosis from the age of 12 years but this had never progressed and on examination he had a minimal thoracic scoliosis to the left; no investigations were undertaken.

II-1 reported that both his parents were unaffected. His father had died at 34 years from TB (date of birth unknown) and his mother at 63 years (date of birth unknown) from a myocardial infarction. II-1 was classified as a new mutation.

II-2 - 6.4.37, 47.2 years. Housewife; SC: II; secondary modern school education, no qualifications.
Examination of skin and iris normal.

III-2 - 19.3.64, 20.2 years. University student; SC: I; grammar school education, achieved 3 'A' level passes.
Examination of skin and iris normal; Ht: 180 (75).

NF 10

II-1 was referred to the study by his GP and also ascertained via the Genetic Clinic records and Dermatology Histology Index.

II-1 - 24.10.47, 36.5 years. Council gardener; SC: IV; secondary modern school education, no qualifications.

4 CAL; freckling: bilateral and around base of neck; CNF: 3; face: no; LN: 17; GNF: 1; Ht: 165 (10); HC:58 (97).

II-1 had not worried about his skin appearance until after his marriage. He was then referred to a dermatologist and the diagnosis made (1 cutaneous neurofibroma was removed to confirm diagnosis). He had subsequently been referred for genetic counselling. He was unwilling for his parents and sister to be reviewed for the present study.

Classified as new mutation.

I-1 - 8.9.22

Had previously been examined in the Genetics Clinic, no abnormality on examination.

I-2 - 3.4.23

No abnormality on examination of skin at Genetics Clinic.

II-2 - 20.1.53.

Reported normal.

III-1 - 16.1.78, 6.3 years.

No abnormality on examination of skin or iris.

III-2 -6.5.81, 3 years.

No abnormality on examination of skin.

NF 11

II-1 was referred to the study by his GP and independently ascertained through Genetic Clinic and general hospital records.

II-1 - 2.1.73, 12.8 years. Schoolboy; SC: III; attending school for physically handicapped children, no intellectual but considerable psychological problems.

12 CAL all >1.5 cm; freckling: axillae; CNF: 1; face: yes; LN: left eye only examined, LN present (unable to count accurately); GNF: 4; Ht: not appropriate; HC: not appropriate.

CAL spots were noted by his parents from birth. Bulging of the right eye was noted at 8 months, paediatric and ophthalmic referral was made. Initially thought to be secondary to congenital glaucoma but on review it became clear the problem was a plexiform neurofibroma of the orbit and the slight increase in intraocular pressure secondary to this. This lesion slowly enlarged although only involved the orbital area. He had been seen by cranio-facial surgeons in two centres and had undergone a number of procedures to reduce the bulk of the tissue. Despite this, at the time of assessment, his right eye was blind, the lesion still large, and eventual enucleation of the whole orbit was planned.

In addition to this, his parents had also noted bowing of the left tibia during the first year of life. At one year he fell and fractured this, and when the fracture failed to heal a diagnosis of pseudoarthrosis related to NF-1 was made. He had a series of unsuccessful corrective procedures and was almost continually in plaster from the age of 1 until his below knee amputation in 1984. When assessed for the study he was having no problems with mobility with his artificial limb.

Classified as new mutation.

I-1 - 9.9.48.

Refused to be seen for study. Had previously been examined in genetics clinic and no NF-1 features noted. His wife reported that he could not accept his son's problems. They had sought a number of second opinions over the years because of this.

I-2 - 6.6.50.

Prepared to answer questions but examination not undertaken. Skin noted to be normal when seen previously at genetics clinic.

II-2 - 7.6.79.

Reported normal.

NF 12

The ascertainment of this family was rather complex! Only the descendants of II-6 lived in the area of the population study; although III-8 is the father of IV-7 and IV-8, he is not in touch with them. III-8 was referred to the population study by his GP and ascertained by medical records, as was IV-8; IV-7 was ascertained via medical records. IV-4 and his branch of the family were ascertained via genetic clinic records for linkage studies and the pedigrees subsequently found to connect (all the children of II-6 had been adopted).

III-8 - 7.1.54, 31.3 years. Building labourer; SC: IIIN; secondary modern school education, no qualifications; experienced difficulties in learning to read and required extra tuition at primary school. 17 CAL; freckling: axillae; CNF: 4; face: no; LN: not examined; GNF: 2; Ht: 170.5 (50); HC: 59.5 (>97).

III-8 was diagnosed during a hospital admission for asthma. He had had several cutaneous neurofibromas removed for cosmetic reasons. His only other complaint was of occasional intense pruritis related to his neurofibromas. He was unaware of the inheritance of NF-1.

IV-7 - 4.10.73, 12.1 years. Schoolgirl; SC: IV; attending comprehensive school, no educational problems.

8 CAL >1.5 cm; 28 >0.5 cm; freckling: axillae and groins; CNF:1; face: no; LN: not examined; GNF: 1; Ht: 141 (10); HC: 54 (75)
IV-7 had also been diagnosed during a hospital admission for asthma. No NF-1 related problems. Mother unaware of implications of diagnosis.

IV-8 - 20.4.77, 8 years. Schoolboy; SC: IV; remedial class education. 8 CAL >1.5 cm; 13 CAL >0.5 cm; freckling: axillae and over trunk; CNF:1; face: no; LN: 24; GNF: 2; Ht: 118.5 (10); HC: 51.5 (25)
Diagnosed at 3 years when referred to a paediatrician for delay in speech development. Swelling over lumbar spine with overlying hypertrichosis noted, spina bifida occulta queried but x-rays normal. On examination for study, the lesion was found to be a plexiform neurofibroma.

IV-4 - 5.2.67, 17.3 years. Student on course for people with learning difficulties; SC: IV; comprehensive school education, required extra tuition with reading. Attained 1 CSE (grade 5).

12 CAL; freckling: axillae and trunk; CNF: 3; face: no; LN: 21; GNF: 2; Ht: 151(3); HC: 57(90).

Diagnosed in 1980 when referred to Growth Clinic for short stature. Endocrine investigations normal (including insulin stress test and usual parameters of pituitary function). Followed in Growth Clinic and growing parallel but below the 3rd centile for height. Family referred for genetic counselling from the Growth Clinic. Had 2 cutaneous neurofibromas removed in 1980 for diagnostic purposes.

I-1 and I-2 - The family did not know who had been affected in generation I. No accurate details were available of age at or cause of death for either I-1 or I-2.

II-1 - Disease status uncertain. Said to have died of a "brain tumour", age unknown.

II-2 - Reported normal

II-3 - 29.12.15, died 64 years. Reported affected by family and confirmed via hospital records. At age of 12 years he had broken his

right leg, and as the fracture did not heal, had had an amputation (no more details available). Died from acute renal failure and a chest infection complicating treatment for a fractured left femur.

II-4 - Reported normal.

II-5 - 2.1.21, 63.4 years. Housewife, SC: IIIM; secondary modern school education, no qualifications.

4 CAL; freckling: axillae and collar-line; CNF: 4; face: no; LN: 13; GNF: 2; Ht: 141 (<3); HC: 54 (50)

Accepted NF-1 as a "family problem" and never sought medical advice, other than for removal of a cutaneous neurofibroma on one occasion. Experienced pruritis over neurofibromas.

II-6 - 16.2.23, died 32 years.

Hospital records reviewed. Definite case of NF-1 with left sphenoid wing dysplasia. Presented at 32 years with 9 month history of headaches and increasing apathy. Investigation showed a right frontal tumour which was partially resected; histology was of a pyeloid astrocytoma. Died in post-operative period, no other lesions identified at autopsy.

III-1 - 5.8.44, 40 years.

Examination of skin normal. Children reported normal.

III-2 - Reported affected by sister. Said to "live like a hermit". Refused to be seen.

III-3 - 22.11.44, 39.5 years. Housewife; SC: III; secondary modern school education, no qualifications.

7 CAL; freckling: axillae and neck-line; CNF: 3; face: no; LN: 100; GNF: 2; Ht: not available; HC: 54 (50)

Unaware of exact cause of skin appearance until seen in genetics clinic with IV-4. Long-standing lower thoracic kyphoscoliosis not investigated until 1976 when presented with dyspnoea and chronic cough (heavy smoker). Radiology of spine had shown dysplastic vertebrae in area of curve, changes reported as being consistent with

NF-1. Chest x-ray showed bilateral emphysema with bullae on the right side.

III-4 - 21.4.48, died at 7 years from Brights disease. Reported unaffected by mother.

III-6, III-7, III-9, III-11 - These children had been adopted following their mother's death and no details were available through the family or the Cardiff adoption agencies. III-6 was thought to have died in a road traffic accident.

IV-3 - 16.6.63, 20.9 years. On Youth Training Scheme; SC: IV; comprehensive school education, attained 1 CSE (grade 5) 10 CAL; freckling: axillae and over trunk; CNF: 2; face: no; LN: 3; GNF: 1; Ht: 157 (3); HC: 57 (97).

IV-5 - 29.3.70, 14.1 years.
Normal examination of skin and iris.

IV-6 - 27.9.74, 11.1 years. Attending comprehensive school, no educational problems.
2 CAL (1 cm diameter) on examination. Slit lamp examination not performed. Classified normal. Ht: 147 (75).

NF 13

Family ascertained via III-1 and III-2, from general hospital records.

III-1 - 19.5.23, died 54 years.

III-1 had been a long-stay hospital patient in a unit for the mentally handicapped prior to his death. His hospital records document features of NF-1 and congenital hydrocephalus, cause uncertain. He was severely retarded and suffered from epilepsy. It is also documented he had kyphoscoliosis but no details are given. At the age of 54 he had an acute haematemesis and died despite resuscitative attempts, source of haemorrhage not established.

III-2 - 12.7.27, 58.1 years. Former lift engineer on invalidity benefit from 1979; SC: IIIM; attended secondary modern school, no qualifications.

7CAL; freckling: axillae and over trunk: CNF: 3; face: no; LN: not examined; GNF: 1; Ht: not appropriate; HC: 59.5 (> 57).

Diagnosed in 1972 when presented with low back pain. Radiological examination had shown Paget's Disease involving the pelvis and femora, posterior scalloping of the lumbar vertebrae and compression fracture of T11 and 12. No active treatment undertaken and intermittent severe back pain since, unable to work because of this. No NF-1 related problems.

II-1 - 14.2.02, 83 years. Long-term patient in psychogeriatric unit. No details regarding education available.

Limited examination: 5 CAL; freckling: axillae; CNF: 3; face: no; LN: not examined.

Although III-2 reported she was affected this had not been formally documented in hospital records prior to study assessment. She had senile dementia, a history of previous strokes, was immobile and confused. She had not had any NF-1 complications. Details of her family were obtained from III-2, who thought his grandparents and mother's siblings were unaffected. The cause of death was known for II-4 and II-5, these were tetanus and a road traffic accident respectively.

Classified as a probable new mutation.

III-3 - Living in New Zealand, reported normal

NF 14

Ascertained via III-1 and III-2 who were referred both by their paediatrician and GP. They had been in foster care from the age of 7 months because of social problems. Their mother (II-1) and her family lived outside the study area.

III-1 - 19.12.74, 10.8 years; schoolboy; SC: IV; remedial class education.

8 CAL >1.5 cm, 4 CAL >0.5 cm; freckling: axillae and groins; CNF: 2; face: no; LN: not examined; GNF: 2; Ht: not appropriate; HC: 51.5 (25).

III-1 and III-2 have been under regular paediatric care from birth which was at 35 weeks' gestation and the diagnosis of NF-1 is mentioned in their notes from the age of 2 years.

III-1 had been noted to have bowing of the right leg when he began walking at one year and was referred for orthopaedic assessment in 1977. X-rays showed marked bowing of both the lower tibia and fibula with cortical thickening and sclerosis of the medial aspect of the lower end of the tibia. The leg had been under observation with no active treatment and no change had occurred to the time of assessment.

Pre-school psychological assessment (4 years 4 months) had shown an IQ of 87 and a mental age of 3 years 10 months. At the time of study assessment he was in a remedial class and having particular problems in reading and writing.

III-2 - 19.12.74, 10.8 years; schoolgirl; SC: IV; remedial class education.

6 CAL >1.5 cm, 5 CAL >0.5 cm; freckling: axillae and groins; CNF: 2; LN: not examined; GNF: 1; Ht: 139.5 (97); HC: 50 (10).

III-2 had a post-axial extra digit on her right hand which had been removed at birth. She had no NF-1 related problems apart from intellectual ones. Pre-school psychological assessment (4 years 4 months) had shown an IQ of 79, mental age 3 years 7 months. She had experienced slightly more problems than her brother in reading and writing to the time of assessment.

II-1 - 21.11.44, 41 years. Catering assistant (unemployed); SC: IV; secondary modern school education, no qualifications.

II-1 had not experienced any major NF-1 problems and had been diagnosed at the age of 15 years when the family became concerned about her appearance. She had a small plexiform neurofibroma, approx. 3 cm diameter on her right cheek. She also had very slight lateral bowing of her left leg, but did not want this X-rayed.

Remainder of family - II-1 reported she was the only affected

individual in her own family. Her father, I-1, had died from right ventricular failure complicating chronic bronchitis and emphysema.

NF 15

II-1 and II-2 were both ascertained by hospital records and individually referred by their plastic surgeon and GP respectively. Because of social problems, the children of I-1 had been in care or adopted from an early age.

II-1 - 23.12.53, 31.5 years. Cleaner (unemployed); SC: V; special school education, now literate and leading an independent life.

16 CAL; freckling: axillae and over trunk; CNF: 3; face: yes; LN: not examined; GNF: 4; Ht: not measured; HC: not appropriate.

II-1 had an extensive plexiform neurofibroma involving all branches of the right trigeminal nerve with relative sparing of the orbit; this had been present from birth. She had had a number of plastic surgical procedures but was still left with severe cosmetic problems and had taken an overdose because of this on one occasion. Her vision was unaffected.

In 1981 she developed grand mal seizures which were well controlled on phenytoin.

She had had 2 miscarriages and 2 stillbirths. Details of one of the latter were available, the foetus was macerated but no abnormalities found and chromosomes were normal.

II-2 - 7.2.57, 28.5 years. Gardener (unemployed); SC: IVM; special school education, now literate and leading independent life.

16 CAL; freckling: axillae and groins; CNF: 3; face: no; LN: 86; GNF: 2; Ht: 170.2 (25); HC: 55.5 (75).

Brought up in children's home from 4 years (as had been II-1). NF-1 was formally diagnosed when he was being investigated for chest pain in 1980 (cause uncertain).

On examination he had a plexiform neurofibroma of the left fourth finger with overgrowth of the finger and overlying CAL pigmentation.

I-1 - 7.11.33, died 55 years.

Reported affected by both II-1 and II-2 independently. Only hospital records available from gynaecology reviews. She had had a total of 14 pregnancies, only 4 of which had resulted in live births. There were no specific details about the remaining 10 in the notes. In 1978 she presented with anorexia, abdominal pain and altered bowel habit. A grade 3 carcinoma of the cervix was found and treatment unsuccessful. There was no mention of NF-1 in her gynaecology notes.

No details of her family are available.

II-3 - 1.8.58, 27.3 years.

Normal cutaneous examination, children reported normal.

II-4 - 19.6.60, 25.6 years. Provisions assistant; SC: III; comprehensive school education, no qualifications. Adopted in early childhood.

7 CAL; freckling: groins; CNF: 2; face: no; LN: 15; GNF: 1; Ht: 154 (10); HC: 53.5 (25).

Diagnosed secondary to study, no NF-1 related problems. Had had one mid-trimester miscarriage.

II-5 - Lived outside study area, thought to be approximately 24 years old in 1985, reported normal by II-4.

III-1 - 2.9.84, 0.9 years. Normal milestones to date of assessment.

11 CAL >0.5 cm; 0 >1.5 cm; freckling: none; CNF: 1; face: no; LN: not examined; GNF: 1; length: 75 (50); HC: 44.9 (25).

Diagnosed secondary to study.

NF 16

II-4 was referred to the study by her GP.

II-4 - 5.9.01, 83.7 years.

She was seen at an old people's home where she had been resident for a number of years. She was demented, her medical and family history was largely supplied by the matron. She had had no major NF-1 problems but at the time of assessment was under review for

post-menopausal bleeding. Her gynaecologist felt investigation was not justified and she was being treated empirically with progesterone and the bleeding had decreased in volume. The matron reported that she complained frequently of pruritis from her neurofibromas.

On limited examination she had multiple cutaneous neurofibromas (grade 4) and axillary freckling.

The matron said that relatives who visited the home reported no history of NF-1; she had asked this specifically in connection with my visit.

Classified as a probable new mutation.

NF 17

II-1 and III-2 were referred by their GP and also ascertained via hospital records.

The other children of II-1 lived outside the study area and were visited during 1987 for the purpose of linkage studies (and were not used for any other analysis).

II-1 - 4.2.05, 80.4 years. Housewife; SC: IIIN; normal schooling, no qualifications.

2 CAL; freckling: none; CNF: 4; face: no; LN: 20; GNF: 3; Ht: 154 (10); HC: 55 (75)

Diagnosed when III-2 sought medical opinion in 1966. She had had a few cutaneous neurofibromas removed for cosmetic reasons and they were intermittently itchy. In 1982 she presented with a 2-year history of abdominal pain and altered bowel habit. Investigation showed a right abdominal mass and at laparotomy a pedunculated neurofibroma was found within the terminal small bowel, with a separate tumour in the wall of the small bowel proximally. A right hemicolectomy was performed and a segmental small bowel resection to remove the proximal tumour. Histology showed benign neurofibromas and in the wall of the resected bowel there was diffuse neuromatous overgrowth with areas of neurofibroma. She had no post-operative problems.

Classified as a new mutation.

III-2 - 28.7.33, 51.9 years. Never worked secondary to neurological problems; SC: IIIN; normal schooling, no qualifications.

13 CAL; freckling: none; CNF:3; face: yes; LN: 74; GNF: 3; Ht: 161.5 (50); HC: 56 (90).

NF-1 was diagnosed in 1966 when she presented with cutaneous neurofibromas, several of which were painful. 3 lesions were removed, histology showed benign neurofibromas. In 1984 she presented with colicky abdominal pain and investigation showed an abdominal mass. At laparotomy a large neurofibroma of the appendix was removed, a few smaller lesions on the serosal surface of the small bowel were noted. Complete resolution of symptoms post operatively. These were the only NF-1 related problems.

Other medical problems

(a) Multiple sclerosis - This had been diagnosed when she was 15 years of age when she presented with an episode of diplopia and loss of use of her right arm and left leg. This had partially resolved and she had had intermittent relapses from that time. Her most recent neurological assessments (in 1979 and 1983) had found few objective clinical signs and considered some of her problems were functional. On examination for the study she had an intention tremor of the right arm, her legs were held stiff but reflexes were normal and her gait was ataxic but much worse when being observed.

(b) Mitral valve prolapse - This was diagnosed in 1979 (on echocardiography) during an admission for assessment of (a); this was asymptomatic.

(c) "Bizarre leiomyoblastoma" of uterus. She had had a hysterectomy for menorrhagia in 1973, histology showed uncomplicated fibroids and in addition one polyp which showed the above histological pattern.

I-1 - Born 1892, died in 1922 from asthma. Reported normal.

I-2 - Born in 1876, died in 1952 from carcinoma of the bowel. Reported normal.

II-2-4 - They and their children reported normal.

II-5 - Reported normal. Died at 23 years from congenital heart disease.

II-6 - He and 2 children reported normal; died at 34 years in an accident.

II-7 - He and 3 children reported normal; died at 80 years from myocardial infarction.

III-1 - 3.2.32, died aged 51 years.

CAL spots noted by mother in early childhood and also a lesion on the left chin. She had a series of operations but was left with residual tissue; presumed plexiform neurofibroma.

In 1972 she presented with paraesthesiae and weakness of the left arm and leg. Myelogram had shown several small filling defects in the upper cervical region. Decompressive laminectomy was performed at C1-7 and the lesions were neurofibromas. One small one was removed for histological confirmation. In 1982 her symptoms recurred, with progressive deterioration and a large neurofibroma was removed at C2. Post-operatively she developed obstructive hydrocephalus and a pseudomeningocele in the scar. A ventriculoperitoneal shunt was inserted. One month later the pseudomeningocele bulged again and was repaired, shortly after this operation she had a cardio-respiratory arrest after which, despite restoration of normal heart rhythm and respiration, she remained unconscious initially. Over the next weeks and months she showed some improvement to the extent of opening her eyes, but it did not appear that she had any comprehension. She eventually died in June 1983.

III-3 - 5.3.37, 50 years

Normal examination of skin.

III-4 - 31.8.40, 47.3 years

Multiple CAL spots; freckling: none; CNF: 4; face: no.

In 1966 had successful removal of a cervical neurofibroma at C2/3 with complete resolution of neurological symptoms. No other NF-1 related problems.

IV-1 - 17.6.60, 27.5 years.

Multiple CAL spots; freckling: axillae, neckline, mammary region and groins; CNF: 3; face: no.

IV-2 - 19.6.61, 26.5 years

Normal examination of skin.

IV-3 - 8.12.62, 24.3 years

Normal examination of skin

IV-4 - 1.12.63.

Reported normal

IV-5 - 1.12.63, 23.3 years.

Multiple CAL; freckling: axillae; CNF: 3; face: no.

IV-6 - 19.5.68, 18.7 years

Multiple CAL; freckling: axillae; CNF: 3; face: no.

Congenital nystagmus and partially sighted, CT scan in the past had not shown evidence of an optic glioma; presumed to be unrelated to NF-1.

IV-7 - 11.7.66, 20.3 years

Normal cutaneous examination.

IV-8 - 19.5.68, 18.5 years

Multiple CAL; freckling: axillae and groins; CNF: 1; face: no; plexiform neurofibroma of right ankle.

NF 18

II-1 and III-1 were ascertained via hospital records, III-1 was also referred by her GP.

II-1 - 22.2.12, died 63 years.

On reviewing his records, NF-1 had been diagnosed incidentally during hospital review in 1945 (age 33 years). From 1970 he was hypertensive but this was well controlled; urinary VMAs were normal on one occasion. He suffered 3 strokes (in 1970, 1974, 1975) and died shortly after the last one.

III-1 knew very little about her grandparents but thought her father was the first case of NF-1 in the family. II-1 was presumed to be a new mutation but excluded from analysis.

III-1 - 4.11.38, 46.6 years. Clerical Officer; SC: IIIN; grammar school education, no qualifications.

16 CAL; freckling: axillae, submammary region and groins; CNF: 3;

face: no; LN: not examined; GNF: 1; Ht: 152.5 (3); HC: 53 (10).

III-1 had been hypertensive for 10 years and was well controlled on atenolol 50 mg per day. She had been reviewed by a neurologist in 1984 for paresthesiae in the right fifth finger, no significant abnormalities were found on examination or nerve conduction studies. Cervical spine x-ray showed spondylosis and it was felt that this was the cause of her symptoms. Her symptoms had persisted intermittently; neurological examination was normal when assessed for study.

III-2 - 5.3.48, 37.7 years. Home care aid; SC: IV; secondary modern school education, no qualifications.

Normal examination of skin. Ht: 165.5 (75). Children reported normal.

NF 19

II-3 was ascertained via hospital records.

II-3 - 31.8.47, 37.8 years. Housewife (former cleaner); SC: IV; secondary modern school education, no qualifications.

11 CAL; freckling: axillae and submammary regions; CNF: 4; face: no; LN: 25; GNF: 2; Ht: 156 (10); HC: 56.5 (97)

Diagnosis had been made in her late teens and she had experienced no major problems. She had had several cutaneous neurofibromas removed when they became particularly large.

On examination the only other abnormality was a right divergent squint with ipsilateral myopia, her fundi were normal.

Classified as new mutation.

I-1 - Birth date unknown, died aged 57 years; cause of death unknown.

I-2 - Age unknown accurately (said to be "in her 70s"); II-2 did not want her contacted. Reported normal.

II-1 - Sister had lost touch with her brother

II-2 - Lived in Australia, no medical details.

Despite the rather vague historical details, II-3 was adamant that neither her parents nor siblings had NF-1.

III-1 - 4.9.67, 17.7 years. Warehouse assistant; SC: IV; comprehensive school education, no qualifications.
Normal examination of skin.

III-2 - 27.8.68, 16.7 years. Unemployed since leaving school; SC: IV; comprehensive school education, no qualifications.
1 CAL spot on examination, irides not examined. Classified normal.

III-3 - 14.4.76, 9.1 years. Schoolboy; SC: IV; attending primary school, no problems.

6 CAL >1.5 cm; 4 CAL >0.5 cm; freckling: no; CNF: 1; face: no; LN: 10; GNF: 1; Ht: 123.5 (10); HC: 54 (25)

His mother first noted one or two CAL spots at birth. Diagnosis was formally made in 1981 when he was referred to a paediatrician with an unrelated problem. He was under regular review to monitor for the development of complications of NF-1. The only other medical consultation had been for a right divergent squint which had been corrected surgically; when assessed for the eye study his eye movements, visual acuity and fundi were normal.

NF 20

Both II-1 and IV-1 were referred to the study by their GP; IV-1 was also referred by his paediatrician and ascertained via hospital records. III-2 lived outside the study area and was examined in 1986 for the purpose of linkage studies (not used in other analyses).

II-1 - 31.8.20, 64.8 years. Factory worker; SC: IV; normal schooling, no qualifications.

4 CAL; freckling: axillae; CNF: 4; face: no; LN: 19; GNF: 1; Ht: 164.5 (75); HC: 56 (90).

II-1 had been diagnosed at approximately 35 years when she became worried about the appearance of her skin. She was on treatment for

hypertension which was well controlled.

On examination she had a plexiform neurofibroma over the left elbow, 6 cm in diameter. She also had slight conductive deafness in the left ear and a cataract in the right eye.

Classified as a new mutation.

IV-1 - 15.8.73, 11.9 years. Schoolboy; SC: IIIM; attending remedial class at comprehensive school.

17 CAL all >1.5 cm; freckling: axillae; CNF: 1; LN: 13; GNF: 4; Ht:153 (50); HC: not appropriate.

IV-1 had been diagnosed on the basis of multiple CAL spots at the age of 4 years. 2 years later he presented with right sided amblyopia (vision 6/60) and disc pallor. CT showed an enlarged chiasm and at craniotomy this was found to be due to an optic glioma (biopsy confirmed), the right optic nerve was also enlarged. The lesion was treated with radiotherapy, his vision improved thereafter and remained stable. Examination when assessed for the study (age 12 years) showed bilateral optic atrophy with 6/12 vision in the right and 6/24 in the left eye.

I-1 - Died 75 years, cause unknown. Reported normal.

I-2 - 13.6.1896, died 87 years, after fracturing a femur. Reported normal.

II-2 - Died in infancy, cause unknown.

III-1 - 25.8.44, 40.9 years. State enrolled nurse; SC: II; secondary modern school education with no qualifications on leaving school but had then completed nursing course.

3 CAL; freckling: submammary region; CNF: 4; face: no; GNF: 2; Ht: 162 (50); HC: 56.5 (97).

Had one cutaneous neurofibroma removed while training and diagnosed at that time.

III-2 - 11.8.47, 39.5 years.

Normal cutaneous examination.

IV-2 - 22.3.76, 9.3 years. Schoolgirl; SC: IIIM; attending primary school, no problems.

6 CAL >1.5 cm; 3 >0.5 cm; freckling: axillae; CNF: 2; face: no; LN: 12; GNF: 3; Ht: 138 (90); HC: 54.5 (97).

CAL spots were noted from birth, formal diagnosis made when brother had eye problems and a plexiform neurofibroma of the left ankle also noted.

On examination at the time of the study, this was a diffuse swelling round the medial aspect of the ankle. Her left foreleg was 2 cm longer than the right and her left foot one shoe size larger.

IV-3 - 18.10.77, 7.7 years. Schoolboy; SC: IIIM; attending primary school, no problems.

Normal examination of skin; Ht: 132 (90).

NF 21

IV-3 was referred to the study by his GP and ascertained through hospital records.

II-2 and IV-1 lived outside the study area, in Mid-Glamorgan.

IV-3 - 30.8.68, 16.7 years. Shop assistant; SC: IV; comprehensive school education, no qualifications.

30 CAL; freckling: axillae; CNF: 2; face: no; LN: 12; GNF: 1; Ht: 180.5 (75); HC: 58.5 (>97).

CAL spots had been obvious from birth and the diagnosis made by the GP. At the age of 10 years he had been investigated by a neurosurgeon for episodic low back pain; there were no neurological signs on examination and myelogram was normal. The pain had resolved with no active treatment. He had a neurofibroma removed from his left posterior tibial nerve and four lesions from his left median nerve at the age of 15 years, with no residual neurological deficit. At the time of study he had no NF-1 related problems although a deep neurofibroma was noted proximal to the scar on his left arm.

Details of generation I and II were obtained from III-2:-

I-1 - Age at and cause of death unknown; uncertain whether he had any NF-1 features.

I-2 - Died 75 years, cause unknown; reported normal.

II-1 - Reported normal; did not reply to letter about the study.

II-2 - Died 70+ years from carcinoma (site unknown). He and son reported normal.

II-3 - Died 70+ years from carcinoma of lung. He and children reported normal.

II-4 - Died from tuberculosis, he and children reported normal.

II-5 - 17.6.05, died 70 years from bronchial pneumonia; also had chronic bronchitis and rheumatoid arthritis. Reported affected by family, this was confirmed on reviewing hospital records.

III-1 - 17.2.36, died 43 years from carcinoma of breast. Reported affected by family, confirmed from hospital notes; had had several cutaneous neurofibromas removed but no other NF-1 related problems.

III-2 - 3.4.30, 55.5 years.

Normal cutaneous examination.

IV-1 - 26.5.63, 32.3 years. Housewife; SC: IVM; remedial class at primary school only; no qualifications.

20 CAL; freckling: axillae, groins, submammary regions; CNF: 3; face: no; LN: 8; Ht: 167 (75); HC: 56 (90).

Had had 2 cutaneous neurofibromas removed for cosmetic reasons, no other problems.

IV-2 - 22.8.64, 20.7 years. Secretary; SC: IIIN; comprehensive school education; obtained 'O' level passes in several subjects and completed a secretarial course on leaving school.

14 CAL; freckling: axillae, submammary region and groins; CNF: 2; face: no; LN: 8; GNF: 2; Ht: 153 (10); HC: 54.5 (50).

Had orthopaedic review at the age of 14 years because of a diffuse swelling over the medial aspect of the left foot. This had been partially excised and histology was of a plexiform neurofibroma. On examination for the study there was residual diffuse swelling under the scar but this was asymptomatic.

V-1- 18.12.83, 1.8 years. Normal developmental milestones.

3 CAL >1.5 cm; 10 >0.5 cm; CNF: 1; face: no; LN: not examined; Ht: 82 (50); HC: 48 (75).

V-2 - 15.5.85, 0.25 years.

Normal cutaneous examination.

NF 22

II-1 was referred to the study by his GP and ascertained through hospital records.

II-1 - 13.9.18, 66.7 years. Retired steel worker; SC: IV; secondary modern school education, no qualifications.

0 CAL; freckling: axillae; CNF: 5; face: yes; LN: not examined; GNF: 3; Ht: 157.5 (<3); HC: 59.5 (>97)

NF-1 had been diagnosed when in hospital for an unrelated problem at the age of 22 years. He had no NF-1 related complications but experienced pruritis from his neurofibromas and was acutely embarrassed by his appearance.

In 1981 (aged 63) had presented with a bloody discharge from his right nipple associated with a breast lump. Investigation showed an intraduct invasive carcinoma of the breast and a Halstead mastectomy was performed; the axillary nodes were involved. He had had a local recurrence in 1982 and was treated with tamoxifen with some success, but it recurred 6 months later and radiotherapy was undertaken. He had then been well until 1985 but at the time of assessment he was having treatment for lung secondaries.

Unable to classify regarding NF-1 inheritance.

I-1 - No details as had left home when II-1 was very young.

II-2 - Died 80+ years, cause unknown. Reported normal. No history of NF-1 in her extended family.

II-2 was referred to the study by his GP.

II-2 - 24.5.52, 33.2 years. Severe mentally retarded, never worked; special school education.

Multiple CAL spots (unable to count); freckling: axillae; CNF: 4; face: no; LN: present but unable to count; GNF: 4; Ht: not measured; HC: 59 (>97).

II-2 had been noted to have CAL spots from the age of one year and cutaneous neurofibroma from 3 years. He had severely delayed developmental milestones and was assessed by a paediatrician at the age of 3 years when the diagnosis of NF-1 complicated by severe retardation was made. There was no suggestion of other causative factors in the perinatal history. No radiological investigations were undertaken and II-2 had not been under regular hospital review from that time. He had attended a special school but since leaving, apart from a short period of attendance at an Adult Training Centre, had been at home.

On assessment for the study he was severely retarded, mental age being subjectively assessed at 2 years. He had a large dolicocephalic skull with posteriorly rotated ears but no other dysmorphic features. He had a plexiform neurofibroma, 4 cm in diameter, in the right lumbar region and he walked with a clumsy ataxic gait, but detailed neurological examination was impossible.

It was felt that his mental retardation was particularly severe for NF-1 but investigations to rule out underlying CNS abnormalities would be of academic interest only. Chromosome analysis on him and his parents was normal.

Classified as a new mutation of NF-1.

I-1 - 6.8.19, 66 years. Retired Tax Inspector; SC: II.

Normal cutaneous examination. Extended family reported normal.

I-2 - 12.5.23, 61.3 years. Housewife. Normal cutaneous examination. Extended family reported normal.

II-1 - 15.2.49.

II-1 was reported normal; he lived in Canada where he worked as an anaesthetist. Neither of his children were reported as having futures suggestive of NF-1.

NF 24

III-5, who lived in the study area, was referred to the study by her GP. Although she supplied the pedigree details she refused contact to be made with the family. Her son, IV-1, had been adopted by III-3 who responded to the newspaper article about the study and was seen although she lived outside the study area.

III-5 - 6.10.46, 38.9 years. Moderately retarded, had never worked; special school education had been recommended to the family but refused and she had attended a secondary modern school; for analysis she has been classified as attending a special school because of her low intellectual level at assessment.

16 CAL; freckling: groins; CNF: 4; face: no; LN: 30; GNF: 3; Ht: 157.5 (25); HC: 56 (90).

From her sister it was apparent that the family had been aware of the diagnosis since childhood but her parents unable to accept her intellectual problems. III-5 was illiterate, extremely anxious and her husband answered most of the questions on her behalf. She had had a cutaneous neurofibroma removed for cosmetic reasons but no other NF-1 problems. At the time of assessment she had had a recent onset of deafness in the right ear with discharge; on examination the deafness appeared to be a conductive loss but she failed to attend for an ENT assessment that had been arranged by her GP.

III-3 - 6.12.35, 50 years.

Normal cutaneous examination. Ht: 161.9 (50).

III-3 supplied the remaining family details.

I-1 and I-2 - No details available. Disease status unknown.

II-1 - 26.3.01, died 48 years. Reported as definitely affected with numerous cutaneous neurfibromas but no other health problem before a fatal subarachnoid haemorrhage. She had a post-mortem and her death certificate states subarachnoid haemorrhage secondary to hypertension, atherosclerosis was given as a secondary problem. No records were available to see whether the hypertension was a long-standing problem or whether it had been investigated.

III-1 - Died in infancy secondary to convulsions.

III-2 - 7.1.33. Reported normal by III-3. Lived in the study area but not contacted.

III-4 - Born 1951, died 19 years.

III-4 had no known medical problems until she presented at 19 years with increasing headache and ataxia. Air encephalogram showed obstructive hydrocephalus and a possible filling defect on the left side of the fourth ventricle. A subtotal resection of a left cerebellar astrocytoma was performed following a shunting operation. She died 2 weeks later of bronchial pneumonia. No mention of CAL spots in her neurosurgical notes and her sister did not know whether she had any NF-1 features, therefore classified as diagnosis uncertain.

IV-1 - 19.5.79, 6.5 years. Schoolboy attending a remedial class. 17 CAL >1.5 cm; 14 >0.5 cm; CNF: 1; face: no; LN: not examined; Ht: 111.6 (10); HC: 53 (75).

IV-1 had been cared for by his aunt since infancy as his parents could not cope. He had not been formally diagnosed until seen for the study. He had an educational assessment at 6 years 1 month and on the Wechsler Intelligence Scale showed verbal IQ 94, performance IQ 68 and full-scale IQ 79.

NF 25

II-8 was ascertained by hospital records.

II-8 - 19.11.30, 54.6 years. Former cleaner on invalidity benefit because of asthma; SC: V; secondary modern school education, no qualifications.

6 CAL; freckling: axillae; CNF: 3; face: no; LN: not examined; GNF: 1; Ht: 155 (10); HC: 54 (50).

II-8 had had recurrent hospital admissions for severe asthma since childhood. NF-1 had been diagnosed clinically at 39 years and a cutaneous neurofibroma removed for confirmation. II-8 thought NF-1 was "just a skin problem" and was unaware of its complications or inheritance.

Classified as new mutation.

I-1 - Born 1885 (approximately), died 60 years from a myocardial infarction. Reported normal.

I-2 - Born 1896, died 69 years from carcinoma of the colon. Reported normal.

II-1-7,9 - II-8 was adamant she was the only affected member in her sibship and was not keen for her sibs to be contacted.

II-5 had died from a myocardial infarction at 40 years of age.

III-1 - 9.9.58, 26.9 years. Data control supervisor; SC: IIIN; comprehensive school education; attained several CSE and one 'O' level pass.

Normal cutaneous examination; Ht: 155.5 (10).

III-2 - 16.6.63, 22 years; receptionist; SC: IV; comprehensive school education, obtained several CSE passes.

16 CAL; freckling: axillae; CNF: 2; face: no; LN: not examined; GNF: 1; Ht: 155 (10); HC: 55 (75).

Multiple CAL spots had been noted by mother in infancy. Formal diagnosis of NF-1 was not made until she was seen for the study.

Ascertained by II-1 who was referred by his GP and identified through the hospital records system.

II-1 - 8.2.48, 37.3 years. Heating fitter (unemployed); SC: IV; secondary modern school education, no qualifications.

2 CAL; freckling: no; CNF: 4; face: no; LN: 2; GNF: 3; Ht: not appropriate; HC: 58 (>97).

The diagnosis of NF-1 had been made at 4 years when he was referred to an orthopaedic surgeon with swelling of the left ankle, this was a plexiform neurofibroma. He had three operations for removal of this during childhood but was still left with residual tissue and overgrowth of the left leg; he had epiphyseal plating at the knee to prevent further growth at the age of 16 years. On examination for the study he had an extensive lesion involving most of his foreleg with overlying skin pigmentation, there was a further area of involvement just below the groin; his left leg was 4 cm longer than the right. The leg swelled during the day and slowed his walking pace slightly. He had 5 cutaneous fibromas removed for cosmetic reasons in 1978.

His other medical problems were apparently unrelated to NF-1. In 1978 he was involved in a road traffic accident and although he retained consciousness, had severe facial injury (no notes available but right temporal skull fracture from history) requiring neurosurgical treatment. In 1980 he developed complex partial seizures and CT showed an area of low density in the right frontal region; the appearance was thought to be compatible with an area of previous infarction. His seizures were reasonably controlled on phenytoin. A repeat scan at time of the study showed no alteration in findings and lesion presumed to be unrelated to NF-1.

Classified as new mutation.

I-1 - 18.6.23, died 58 years as a result of an industrial accident. Reported normal by I-2.

I-2 - 8.6.20, 65 years. Normal cutaneous examination.

II-2 and II-3 - 27.6.50 and 3.9.52 respectively. They and their

children reported normal by I-2 who was reluctant for them to be contacted for the study.

NF 27

II-1 was referred to the study by his GP.

II-1 - 20.10.21, 64.1 years. Retired grocer; SC: III; attended elementary school, no qualifications.

13 CAL; freckling: axillae and groins; CNF: 4; face: yes; LN: present, not counted; GNF: 3; Ht: 158 (<3); HC: 58 (>97).

From the time of his parents' death, II-1 had been unable to cope with the family business which had been sold and he lived as a semi-recluse from then. The family history was supplied by II-2 as the propositus was a rather vague historian. The family were aware of the diagnosis in II-1 from childhood, the GP having made the diagnosis. He had had no major NF-1 problems and had only had 1 cutaneous neurofibroma removed from his scalp. He had avoided sporting activities because of his appearance.

Classified as a new mutation.

I-1 - 10.1.92, died 86 years from chronic chest disease (pathogenesis uncertain). Reported normal.

I-2 - 19.10.92, died 83 years from heart failure. Reported normal. There was no history of NF-1 in the extended families of I-1 and I-2.

III-2 - 25.6.25, 60.3 years. Hotelier; SC: II.

Interviewed, not examined; no abnormality reported in himself or children.

III-3 - She and her children reported normal. No reply to letter regarding study.

II-2 was ascertained via the Dermatology Histology Index.

II-2 - 10.6.59, 26.3 years. Shop assistant; SC: IV; comprehensive school education, obtained several CSE passes.

6 CAL; freckling: groins; CNF: 3; face: no; LN: 12; GNF: 1; Ht: 154 (10); HC: 53 (10).

Diagnosis of NF-1 had been made at 22 years when referred to a dermatologist. Four cutaneous neurofibromas had been removed. II-2 had no idea of the genetic implications of NF-1 or its complications. Classified as new mutation.

I-1 - 4.6.34, 51.4 years. Normal examination of skin and iris. No history of NF-1 in extended family.

I-2 - 25.12.31, 53.8 years. Normal examination of skin and iris. No history of NF-1 in extended family.

II-1-3,6 - The siblings of II-2 were reported normal.

III-1 - 23.12.79, 5.8 years. Schoolboy; no developmental or intellectual problems to date.

7 CAL >1.5 cm; 4 >0.5 cm; freckling: none; CNF: 1; face: no; LN: examination not possible; GNF: 2; Ht: 115 (10); HC: 52 (50).

III-1 was diagnosed secondary to the study. On examination he had a small plexiform neurofibroma with overlying pigmentation on his right forearm.

III-2 - 7.9.85, 0.1 years.

One possible CAL spot noted on anterior chest, classified as equivocal.

II-1 was referred by her GP, III-3 ascertained via hospital records and IV-1 and 2 by their paediatrician.

II-1 - 6.2.32, 53.8 years. Housewife; SC: IVM; no details of type of school attended available. Her husband reported she had been kept away from school much of the time as a child; on subjective assessment she was illiterate but had coped with bringing up her family reasonably well - for the purpose of analysis she was classified as requiring special school education but minimally retarded.

12 CAL; freckling: axillae and groins; CNF: 4; face: no; LN: not examined; GNF: 4; Ht: 152 (3); HC 53.5 (25).

At the time of assessment, II-1 was a hospital in-patient and much of the history was supplied by her husband. She had cutaneous neurofibromas from the time he knew her but her only medical problem prior to the 1985 admission had been grand mal epilepsy which she developed in her early 20s. This had not been investigated and she was well controlled on phenytoin. On examination for the study, the only other NF-1 related finding on her skin was a plexiform neurofibroma in the left groin, approximately 15 cm in diameter.

In August 1985 she had presented with symptoms and signs of small bowel obstruction. At emergency laparotomy an ileocolic intussusception was found and right hemicolectomy performed. She had a stormy post-operative period with several hypertensive crises and investigation showed bilateral phaeochromocytomas. Bilateral adrenalectomy was subsequently performed and a peri-ampullary carcinoid lesion was identified and removed at the same time (this had been specifically looked for in view of the known association). She was eventually discharged on 18th December but was readmitted later in the same month with a urinary tract infection. She was responding satisfactorily to antibiotics and steroids, then had a cardiac arrest from which she could not be resuscitated. The cause of death at coroner's post mortem was given as coronary artery disease.

Unable to classify regarding inheritance of NF-1.

III-3 - 22.3.57, died 23 years from neurofibrosarcoma.

III-3 had no NF-1 related problems until she presented with severe pain secondary to a large paraspinal mass in the lumbar region at the age of 23 years. A myelogram was normal. The mass was removed and

histology showed a neurofibrosarcoma. She had a course of palliative radiotherapy but died six months later from complications of tumour involvement of the spinal cord.

IV-2 and IV-3 had been in foster care for most of their life as 2 years after their mother's death their father committed suicide.

IV-2 - 12.2.78, 7.7 years. Schoolboy, attending remedial class.
13 CAL >1.5 cm; 14 >0.5 cm; CNF: 1; face: no; LN: not examined; GNF: 2; Ht: 116.5 (10); HC: 52.5 (50).

Diagnosed in early childhood on the basis of multiple CAL spots and under regular paediatric follow-up. No NF-1 related problems other than in schooling.

IV-3 - 14.11.79, 5.9 years. Schoolboy, attending remedial class.
7 CAL >1.5 cm; 4 CAL >0.5 cm; CNF: 1; face: no; LN: unable to examine; GNF: 2; Ht: 112.5 (25); HC: 53 (75).

Diagnosis of NF-1 had been made at 1 year on the basis of multiple CAL spots and he had been under paediatric care from that time. A recurring problem was of diarrhoea with intermittent abdominal pain for which no cause had been identified (including investigations to exclude a duodenal carcinoid). On examination he had a small 3 cm diameter plexiform neurofibroma under the left nipple.

I-1,I-2,II-2-5 - The husband of II-1 thought that II-4 was possibly affected but did not know any details regarding his parents-in-law. They had lost touch with the parents and siblings of II-1.

III-1 - Died at approximately 6 years after falling into a fire. Her father did not remember her having CAL spots.

III-2 - 12.7.53, 32.2 years. Comprehensive school education, no qualifications. Normal cutaneous examination.

III-4 - 27.11.58, 26 years. Comprehensive school education, no qualifications. Normal cutaneous examination.

III-5 - 8.12.59, 25 years. Comprehensive school education, no

qualifications. Normal cutaneous examination.

IV-1 - 26.5.74, in foster care in West Wales and under regular paediatric review. No features of NF-1 reported to be present by her consultant.

NF 30

IV-1 was referred to the study by her GP and consultant dermatologist.

IV-1 - 13.3.64, 21.5 years. Nursing auxillary; SC: II; comprehensive school education, obtained several CSE passes.

10 CAL; freckling: none; CNF: 3; face: no; LN: not examined; GNF: 4; Ht: 152.4 (10); HC: not appropriate.

Her mother had noted multiple CAL spots as a baby but not been aware of their implication until IV-3 presented with scoliosis and was diagnosed.

IV-1 had no problems until she presented for neurological investigation following 2 grand mal fits (aged 17 years). She had also complained of occasional mild headaches which had been more frequent in the previous six months. On investigation her multiple CAL spots were noted, skull x-ray showed evidence of chronically raised intracranial pressure and CT severe hydrocephalus of the lateral and third ventricles with normal sized fourth ventricle. Diagnosis of aqueduct stenosis complicating NF-1 was made. A ventriculo-peritoneal shunt was inserted and from that time she had had no fits or headaches. She had no other NF-1 related problems and was 17 weeks into her first pregnancy at the time of assessment.

I-1 and I-2 - Both died at 80+ years, cause unknown. Not thought to be affected.

II-1 - Age at and cause of death unknown. Reported normal.

II-2 - 13.3.01. Died 82 years of bronchopneumonia, had senile dementia. Reported affected by family and "covered in lumps". No

mention of NF-1 in geriatric notes.

III-1 - 14.7.34, 51.3 years. Shop cashier; SC: IIIN; secondary modern school education, no qualifications.

Face: yes; GNF: 3; Ht: 164 (10); HC: 58 (>97).

III-1 refused examination saying "it's obvious from my face what's wrong". He had about 40 cutaneous neurofibromas on his face and his only worry regarding NF-1 was his cosmetic effect. He had never sought medical advice about the disease.

III-2 - 26.11.38, 46.8 years. Nursing auxillary; SC: II; grammar school education, obtained school certificate. Lived outside study area.

10 CAL; freckling: none; CNF: 4; face: no; LN: not examined; Ht: 154 (10); HC: 56 (90).

Vaguely aware of diagnosis from childhood; diagnosis was made formally when IV-3 presented with scoliosis. When she became pregnant after this she had a termination because of the risk of NF-1 to the foetus.

III-3 - 28.1.41, 44.7 years. Long-distance lorry-driver; SC: IIIM; secondary modern school education, no qualifications.

Normal cutaneous examination; Ht: 165 (10).

IV-2 - 8.4.66. Reported normal, lived outside study area.

IV-3 - 15.9.68, 16.1 years. On Youth Training Scheme; comprehensive school education, no qualifications. Recently moved to live with III-1.

7 CAL; freckling: none; CNF: 3; face: no; LN: not examined; GNF: 3; Ht: not appropriate; HC: 56 (75).

CAL spots had been noted by his mother at one year. From 10 years he experienced intermittent pain over the thoracic spine and a scoliosis developed. On orthopaedic assessment at 12 years he was found to have "a short sharp 85° right thoracic scoliosis", with a trapezoid shape vertical body at its apex. He had no neurological signs, a myelogram showed narrowing of the canal at the apex of the curve and bow stringing of the cord across the convexity of the apex. He was

initially treated with bracing and at 15 years had a spinal fusion *in situ*, no attempt being made to improve the curve because of the risk of neurological damage. Apart from occasional pain, he had no problems following surgery, but had a marked residual kyphoscoliosis.

On examination the only other feature was a 6 cm diameter plexiform neurofibroma with overlying skin hypertrophy over the sacrum.

NF 31

II-4 was referred to the study by his GP and ascertained via hospital records.

II-4 - 19.9.56, 28.9 years. Barman/cook; SC: IV; secondary modern school education, no qualifications.

7 CAL; freckling: none; CNF: 4; face: no; LN: 12; GNF: 4; Ht: 169.3 (25); HC: 61 (>97)

Although his mother was aware of multiple CAL spots from early childhood he did not come to medical attention until he presented at the age of 20 years with enlargement and pain of several subcutaneous lesions on his right arm. Diagnosis of NF-1 was made and 4 neurofibromas removed from the medial cutaneous nerve of the arm. During this admission his blood pressure was noted to be 210/140, with grade II hypertensive retinopathy. Investigation of this showed a left renal artery stenosis on arteriogram. He was conservatively treated for 2 years but as his blood pressure was difficult to control, was re-investigated in 1978 and a 50% stenosis demonstrated; he had successful saphenous vein bypassing of the left renal artery and required no anti-hypertensive medication post-operatively.

In 1984 he had a painful neurofibroma removed from his left posterior interosseous nerve with damage to the nerve; this was partially corrected by a subsequent tendon transfer. On examination for the study he had grade IV power in the finger and wrist extensors.

Classified as new mutation.

I-1 - 18.9.21, 64 years.

Normal cutaneous examination. No history of NF-1 in extended family.

I-2 - 26.11.21, 63.8 years.

Normal cutaneous examination. No history of NF-1 in extended family.

II-1-3 - They and their children reported normal by parents.

NF 32

II-2 was referred to the study by her GP.

II-2 - 8.7.72, 12.9 years. Schoolgirl attending special school.

13 CAL >1.5 cm; 9 >0.5 cm; freckling: axillae; CNF: 2; face: no; LN: not examined; GNF: 3; Ht: 138.5 (<3); HC: 55 (90).

II-2 had delayed milestones in early childhood, her speech being particularly slow in developing and attended a special school from 5 years. The diagnosis of NF-1 was made at 10 years when she was referred to a paediatrician because of a swelling on her left wrist. Multiple CAL spots were noted with 1 hypopigmented patch; a soft, poorly-defined swelling on the left radius was noted, bones underlying being normal on x-ray and a diagnosis of NF-1 with a plexiform neurofibroma made. No investigation into other causes of retardation were undertaken.

On examination for the study, the left wrist lesion was approximately 6 cm in diameter with overlying hypertrophy and CAL pigmentation; she had a second plexiform lesion on the left thigh anteriorally with overlying skin hypertrophy and hypertrichosis. Her speech was very slow and her gait clumsy but with no local neurological signs. She was classified as moderately retarded on subjective assessment.

Classified as new mutation.

I-1 - 31.1.46, 39.5 years.

Normal cutaneous examination. No history of NF-1 in extended family of him or his wife.

I-2 - 5.1.50, 35.6 years.

Normal cutaneous examination. She had miscarriages in 2 pregnancies at 3 months and 5 months, for which there had been no obvious cause.

II-1 - Had been born prematurely at six months' gestation and died at 18 hours.

II-3 - 22.9.78, 7.2 years.

On cutaneous examination had no CAL spots but one area of patchy coffee-coloured pigmentation over the fourth and fifth toes of the left foot. Classified normal. Ht: 116 (25).

II-3 had bilateral club feet at birth requiring several corrective operations. He had delayed milestones and attended a special school but had had no investigations for his retardation.

NF 33

IV-4 was referred by his GP and ascertained via orthopaedic records.

IV-4 - 23.6.59, 26.1 years. Cook/waiter; SC: IV; comprehensive school education, no qualifications.

17 CAL; freckling: axillae and groins; CNF: 2; face: no; LN: 43; GNF: 3; Ht: not appropriate; HC: 59 (>57).

His mother said that he had multiple CAL spots from the first year of life but the diagnosis was not made formally until he was referred for investigation of nocturnal enuresis in 1974. An IVP showed a thoracic scoliosis but no other abnormality. The scoliosis progressed and he was referred for orthopaedic assessment in June 1975 (aged 16 years); he had a right thoracic scoliosis T8-12, 66°, with no underlying bony abnormality. This progressed to 95° by May 1976 and in July he had a posterior spinal fusion. The curve was corrected to 56° and has remained stable since. No other NF-1 problems.

The family history was mainly supplied by III-2 on telephone interview as she lived outside the study area.

I-1, I-2, II-2-7 - No accurate details available but not thought to have had NF-1.

II-1 - 20.4.09, died 66 years from myocardial infarction. Reported as definitely affected by III-2. No known NF-1 complications.

III-1 - 22.8.31.

Lives in Norway. Reported normal.

III-2 - 9.2.35, died 49 years from lung cancer. Emigrated to USA. Reported definitely affected with no NF-1 complications.

III-3 - 20.12.36. Interviewed on telephone. Reported CAL spots from childhood and cutaneous neurofibromas from 12 years. Reviewing her hospital notes, she had been formally diagnosed by a dermatologist (aged 33 years) when she had had 3 cutaneous lesions removed. She reported no NF-1 complications.

IV-1 - Reported affected by aunt, said to have NF-1 with a related problem "with one of his legs".

IV-2 - Reported normal.

IV-3 - 1.4.57, 28.4 years. Shop assistant; SC: IV; comprehensive school education, no qualifications.
Normal cutaneous examination.

IV-5 - 10.1.65, 20.8 years. Medical student; SC: I; comprehensive school education, attained 3 'A' level passes.
1 CAL spot on cutaneous examination; classified normal.

V-1 - 23.10.78, 6.9 years.

3 very faint CAL spots on chest, 2 >1.5 cm diameter; classified normal.

III-3 was referred by her GP and ophthalmologist.

III-3 - 3.3.67, 18.7 years. School cleaner; SC: V; comprehensive school education, no qualifications.

III-3 answered the study questionnaire but refused full clinical examination, the only recordings were face: no; LN: 4; GNF: 3; Ht: 166.3 (75); HC: 60.5 (>97).

III-3 had presented to an ophthalmologist at 2.5 years with left buphthalmos and goniotomy was performed. Despite this, the intraocular pressure remained elevated and she had 3 further goniotomies within the next 3 years but the pressure gradually rose after each procedure and her vision began to deteriorate. She was referred to Moorfields Eye Hospital for a second opinion when her multiple CAL spots and family history were noted; the left disc was pale with a large cup and the iris flat and featureless. On the nasal angle of the eye there were signs of the previous operations but on the temple side of the iris the trabecular meshwork was covered by a network of pigment. It was felt that this represented a congenital anomaly like that seen in cases of NF-1 complicated by glaucoma. Conservative treatment was advised with cyclocryotherapy if further operations were necessary. The latter was performed in 1979 and her pressure had been normal from then although her vision remained reduced at 2/60.

III-3 had no other NF-1 related problems.

The family details were supplied by II-3.

I-1 - Born 1896, died 83 years, cause unknown; reported normal.

I-2 - Born 1896, died 88 years, cause unknown; reported normal.

II-1 - Date of birth unknown. Lived outsidest study area, reported normal.

II-2 - 2.9.21. Lived in Cardiff but family unkeen for her to be contacted. Reported normal.

II-3 - 25.7.23, 62.3 years. Signalman; SC: IIIM; elementary school education, no qualifications.

2 CAL; freckling: none; CNF: 5; face: yes; LN: 13; GNF: 3; Ht: 162.2 (3); HC: 60.5 (>97).

II-3 knew that he had NF-1 but was not aware of its implications until seen for the study. He had been aware of CAL spots and 2 large masses (plexiform neurofibromas on examination) since childhood, one on his neck and one just below his right breast. He had had the neck lesion partially excised at 19 years (no records available). Cutaneous neurofibromas had begun to appear at 13 years.

On examination for the study he had a scar over the posterior aspect of his neck with diffuse underlying swelling and the lesion on his chest was approximately 10 cm in diameter with overlying skin hypertrophy.

Classified as new mutation.

III-1 - 6.5.58, 27.5 years. State Enrolled Nurse; SC: II; comprehensive school education; obtained several 'O' level passes. III-1 lived just outside the study area. Cutaneous examination was normal; Ht: 167 (75).

III-2 - 23.8.62. Reported normal by his father; lived in the study area but did not reply to letter about the study.

NF 35

II-3 was ascertained via hospital records.

II-3 - 2.3.59, 26.7 years. Office clerk; SC: IIIN; comprehensive school education, no qualifications.

15 CAL; freckling: axillae; CNF: 3; face: no; LN: 35; GNF: 1; Ht: 160.5 (50); HC: 54 (50).

The diagnosis had first been made when her mother sought paediatric review because of multiple CAL spots. She was told the name of the disease and reassured it was not a cause for major worry; no follow-up was arranged. In 1978 II-3 had 2 cutaneous neurofibromas removed because of their unsightly appearance but had no other NF-1

problems. She was 4 months pregnant at the time of assessment and unaware of the inheritance of NF-1.

Classified as new mutation.

I-1 - 23.6.34, 51.4 years

Normal cutaneous examination. No history of NF-1 in extended family.

I-2 - 5.5.36, 49.6 years.

Normal cutaneous examination. No history of NF-1 in extended family.

II-1,2,4 - Reported normal by parents.

NF 36

III-1 was ascertained via hospital records. III-6, who lived outside the study area, was referred by Professor M. Laurence when he attended for genetic counselling.

III-1 - 9.2.48, 37.9 years. Registered disabled, former hospital kitchen assistant; SC: V; secondary modern school education, no qualifications.

7 CAL; freckling: axillae, submammary regions, groins; CNF: 4; face: no; LN: 3; GNF: 2; Ht: 151 (3); HC 54.5 (50).

III-1 was a very anxious lady who had been known to have NF-1 for a number of years (from hospital records) but who was not aware of this or its implications. No major NF-1 problems and had had a few cutaneous neurofibromas removed for cosmetic reasons.

She said she had been registered disabled because of poor vision and right-sided deafness, both of which dated from childhood. ENT notes recorded recurrent treatment of right-sided otitis externa, a narrow external canal being the only abnormal recorded feature.

On examination she had a left convergent squint, her vision was 6/9 in the right eye and 6/18 in the left, which corrected (with pinhole) to 6/6 and 6/9 respectively. She did appear to have a right sensorineural deafness but failed to attend a neurogenetic clinic appointment for review of this.

She denied a family history of NF-1 and was unwilling for her family (who all lived outside the study area) to be contacted. When III-6 was seen in the genetic clinic he gave a different account and this proved to be correct from discussions with the family GP and reviewing hospital records. Despite this the remaining family members were not contacted for the study in respect of III-1's wishes.

III-6 -15.5.55, 30 years. Normal cutaneous examination.

II-4 - 11.1.16, died 68 years from aortic valve disease.

Reported to be the first case of NF-1 in the family; no accurate details about his parents and siblings available. Hospital records from 1982 document multiple cutaneous neurofibromatosis. At that time he was assessed for dyspnoea and a chronic productive cough; examination showed aortic stenosis with incompetence, blood pressure was 180/90, with evidence of heart failure on treatment with bumetidine K and phyllocontin.

Investigations were as follows - chest x-ray: prominent left ventricle, suspicion of background pneumoconiosis with some bronchiectasis; ECG: left ventricular hypertrophy and strain; echocardiograph: calcified tricuspid aortic valve with severe stenosis, fine fluttering of mitral valve being seen as evidence of aortic incompetence, the left ventricle was markedly dilated with left ventricular hypertrophy and a large end systolic volume. In October 1984 he died suddenly at home; after post mortem his cause of death was shown to be calcified aortic valve disease with unrelated pulmonary fibrosis and bronchiectasis.

III-2 - 7.11.49. Reported affected by III-6 and this was confirmed from hospital records.

III-2 had a long history of recurrent duodenal ulceration compounded by heavy alcohol intake. In November 1982 (age 33 years) he was admitted with a haematemesis, endoscopy showed gastritis and a duodenal ulcer; this settled with conservative treatment. His admission notes document multiple CAL spots and cutaneous neurofibromas. He also complained of an enlarging painful mass on the posterior aspect of his left forearm. This was subsequently

removed and found to be an 8 x 5 cm mass arising from the radial nerve. Histology showed a neurilemmoma with low grade malignancy; radiotherapy was not advised. He was kept under review and recurrence had not been observed to the time of the study.

III-3 - 16.3.50. Reported normal.

III-4 - 14.5.51. She and children reported normal.

III-5 - 15.5.55. Reported affected. Had attended a special school and suffered from epilepsy from the age of 10 years.

III-7 - 13.5.62, Reported normal.

IV-1 - 27.1.71, 14.8 years; schoolboy attending comprehensive school. Normal examination of skin and iris.

NF 37

II-16 was ascertained via hospital records.

II-16 - 20.10.27, 58.1 years. Storeman; SC: V; elementary school education, required extra tuition until 12 years of age; classified as requiring remedial class education.

9 CAL; freckling: no; CNF: 4; face: yes; LN: 1; GNF 2; Ht: 169.2 (25); HC: 56.5 (90).

NF-1 had been incidentally diagnosed during an admission for a pneumothorax in 1981; he had had no hospital assessment specifically for NF-1.

Classified as new mutation.

I-1 - Born 1880, died 62 years from myocardial infarction. Reported normal.

I-2 - Born 1884, died 84 years, cause unknown. Reported normal.

II-1-15 - The propositus was the youngest of 15 children and was adamant no one else in the family was affected. He was vague about the ages and causes of death of those siblings who had died.

NF 38

III-2 was referred by his GP and ascertained via hospital and genetic clinic records. II-2 was prepared for her and the 2 children to answer questions but not to undergo examination.

III-2 - 29.8.66, 19.3 years. Had not worked since leaving school; SC: V; special school education, classified as minimally retarded. Face: no; GNF: 3.

The diagnosis of NF-1 was made in 1975 (age 9 years) when he presented to a paediatrician for assessment of grand mal fits. He had had delayed motor and intellectual milestones, requiring special school education. His seizures were grand mal and apart from multiple CAL spots no abnormalities were found on examination. His IQ was 76. An EEG was reported to be non-specifically abnormal and an isotope brain scan was normal. He was treated with phenytoin and ospolot and his seizures were well controlled. On leaving school he had been literate but had never worked. He was classified as minimally retarded as with different social circumstances he would probably have achieved much more.

I-2 - 2.1.07, died 61 years from leukaemia. Reported affected by daughter, no hospital records or details of type of leukaemia traced. No antecedent family history available.

II-1 - Reported normal by II-2 who was unwilling for her siblings to be contacted.

II-2 - 29.11.34, 51.1 years. Housewife; former brewery worker; SC: V; special school education, classified as minimally retarded. Face: no; GNF: 3.

Had never had hospital assessment for NF-1 other than being examined in the genetics clinic on one occasion when the diagnosis was

confirmed. She could not read and could only write her name but as she managed to live independently with the 2 children was classified as minimally retarded for analysis.

II-3 - Reported normal by II-2.

III-1 - 23.9.63, 22.2 years. Had not worked since leaving school; SC: V; special school education; classified as minimally retarded. Face: no; GNF: 3.

At 9 months presented with attacks of salaam spasms and EEG showed typical hypsarrhythmia with had completely responded to prednisolone. Delayed motor and intellectual milestones had followed and she had attended a special school; on leaving was able to read and write. In 1980 she developed grand mal seizures and was well controlled on carbamazepine when assessed for the study. Her mother had been aware of a swelling on the left ankle from early childhood and by 16 years she was unable to fit into normal shoes. She had 3 partial excisions of a plexiform neurofibroma over the next 6 years but when seen there was still an obvious residual mass.

NF 39

III-2 was ascertained via hospital records.

III-2 - 29.11.63, 22.1 years. Attending college course for people with learning difficulties; SC: unable to classify; special school education, classified as minimally retarded.

10 CAL; freckling: axillae and trunk; CNF: 3; face: no; LN: 50; GNF: 3; Ht: 167.5 (10); HC: 57.5 (97).

Although he had required special school education no hospital paediatric assessment was known to have been made in childhood. When seen he was an anxious rather inadequate personality but was literate. He was not aware of the diagnosis of NF-1 though this had been made when he had had a neurofibroma removed from his right ulnar nerve at 13 years.

On examination he had a 6 cm diameter plexiform neurofibroma with overlying skin hypertrophy to the right of his umbilicus.

I-1 - Age unknown. Reported normal by son who was unwilling for him to be contacted.

I-2 - Birth date unknown, died 60+ years, cause unknown. Reported normal.

II-1-3 - Reported normal, II-4 unwilling for them to be contacted.

II-4 - 23.12.34, 51.1 years. Fork lift truck driver (unemployed); SC: IIIM; secondary modern school; no qualifications.

3 CAL; freckling: no; CNF: 3; face: no; LN: 20; GNF: 1; Ht: 160 (3); HC: 58 (97).

Diagnosed secondary to study, no NF-1 related problems. Suffered from angina.

III-1 - 12.10.70. Died 2.5 years from bronchopneumonia complicating spastic quadraplegia with epilepsy which was said to be caused by prematurity. No CAL spots reported by parents.

III-3 - 26.12.64. Reported normal, unable to contact.

III-4 - 21.5.74. Reported normal. Mother unwilling for her to be examined.

NF 40

II-3 was ascertained via hospital records, he was unwilling to be seen for the study but allowed his GP to supply information.

II-3 - 2.5.22, 63.1 years. Barman; SC: IV

Face: yes.

The diagnosis of NF-1 was made in 1959 by a consultant dermatologist when he had a cutaneous neurofibroma removed. He appeared to have had no other NF-1 complications. Several of the author's colleagues frequented the pub in which he worked and reported his face to be covered in neurofibromas.

His GP stated his father was affected but knew no other details

of his case and GP thought that I-1 and I-2 were unaffected.

NF 41

This family was ascertained by III-1, referred by her GP, and IV-1 referred by the GP, his hospital consultant and identified through hospital records.

III-1 - 17.10.44, 40.6 years. Housewife; SC: III; secondary modern school education, no qualifications.

14 CAL; freckling: axillae and groins; CNF: 4; face: yes; LN: 56; GNF: 3; Ht: 153 (10); HC: 56.5 (97).

NF-1 had been diagnosed during her first pregnancy. She had no major complications and her only complaint was of pruritis. She thought that NF-1 was just a "skin problem". She had several cutaneous neurofibromas removed for cosmetic reasons.

On examination she had 2 small plexiform neurofibromas, one on the medial aspect of the left buttock and another on the right ankle.

IV-1 - 2.1.67, 18.4 years; unemployed; SC: III; remedial class education; no qualifications.

19 CAL; freckling: axillae; CNF: 3; face: no; LN: 80; GNF: 2; Ht: 157.5 (<3rd); HC: 58 (97).

Although his mother was aware of the CAL spots during childhood, formal diagnosis was not made until 16 years of age when he was referred for assessment of short stature and lack of pubertal development. Endocrine investigation showed no abnormality and pubertal changes began at 17 years of age. His height was growing parallel to the 3rd centile.

II-8 - Born 1918, died 1949 aged 31 years.

Reported affected by husband. Her main disease related problem had been a large swelling on the left arm which had required surgery during childhood (no records available, ? plexiform neurofibroma). She had died of pulmonary infarction following childbirth.

Although details regarding her parents and siblings are limited, her husband was sure that she had been the first case in the family.

Presumed new mutation but excluded from analysis because of limited details.

III-2 - 3.7.42, 44.2 years. Lived outside study area.

Visited for purpose of linkage studies in September 1986. Brief examination confirmed multiple CAL spots and cutaneous neurofibromas. No major disease complications.

III-3 - Born 1949, died at six months. Described as a 'blue baby'. No other details available.

IV-2 - 19.6.70; 15 years; attending comprehensive school, no educational problems.

Normal cutaneous examination.

IV-3 - 15.10.77; 8.9 years.

Normal cutaneous examination.

NF 42

II-6 was referred to the study by her GP.

II-6 - 14.4.40; 45.6 years. Unemployed chicken sexer; SC: IVM; elementary school education, no qualifications.

7 CAL; freckling: axillae, groins and submammary regions; CNF: 4; face: no; LN: not examined; GNF: 2; Ht: 149.5 (<3); HC: 56 (90).

Although II-6 had heard the disease mentioned on hospital visits, she knew nothing about NF-1 or its genetic implications. From her hospital records the diagnosis had been made during investigations for chest pain at 43 years. She had no disease complications apart from a small plexiform neurofibroma on her abdomen with hypertrophy of the overlying skin.

Classified as new mutation.

I-1 - 13.12.06, died November 1985 aged 78 years.

Had a myocardial infarction and cerebrovascular accident in 1985 following which he became increasingly confused. Examined for signs

of NF-1 during terminal admission at the author's request and reported normal.

I-2 - Date of birth unknown. Died aged 74 years in 1982.

Reported normal by II-6. Suffered from angina and diabetes, cause of death unknown.

Siblings of II-6 - II-6 said she was the only affected member of her sibship. Her siblings and their children were in good health apart from II-1 who had been treated for carcinoma of the breast, and II-9 who was described as 'spastic', being unable to walk or talk. His twin, II-8, had died at 6 months of age, cause unknown.

III-1 - 2.8.58. Lived outside study area.

He and his offspring were reported to be unaffected.

III-2 - 17.10.60. Lived outside study area.

He and his offspring were reported to be unaffected.

III-3 - Born 1961. Reported to have died at 1 month although his mother was uncertain as to the cause, apart from the fact that he had been a poor feeder and was said to have had a large heart.

III-4 - 23.8.65; 20.2 years. Unemployed; SC: IVM; comprehensive school education, no qualifications.

14 CAL; freckling: axillae, groins and submammary regions; CNF: 2; face: no; LN: not examined; GNF: 2; Ht: 152 (3); HC: 55 (75).

III-3 had never been reviewed for NF-1. Mother had noticed CAL spots from infancy and a swelling on the left wrist from 6 years. On examination this was a small plexiform neurofibroma.

III-5 - 26.3.67; 19.7 years; on Youth Training Scheme; SC: IV; remedial class education, no qualifications.

18 CAL; freckling: axillae and groins; CNF: 2; face: no; LN: not examined; GNF: 2; Ht: 173 (50); HC: 56.9 (90).

The diagnosis of NF-1 was formally made when referred for assessment of gynaecomastia at the age of 19 years. No underlying abnormality was found although it was noted that he was still undergoing pubertal

development. Apart from late puberty he had no other NF-1 complications.

III-6 - 17.10.68; 17.1 years; on Youth Training Scheme; comprehensive school education, no qualifications.

Normal cutaneous examination. Ht: 168 (25); HC: 54.5 (50).

III-7 - 16.7.70; 14.9 years; attending comprehensive school; SC: IVM.

9 CAL; freckling: axillae and groins; CFN: 2; face: no; LN: not examined; GNF: 1; Ht: 155 (10); HC: 55.5 (90).

III-7 had never been formally assessed for NF-1 although CAL spots were noted in her hospital records from 7 months of age. She had been under orthopaedic review when younger for bilateral calcaneovalgus deformity and had required lengthening of the left Achilles tendon at 6 years.

On examination, the only other finding of note was a possible plexiform neurofibroma on the right chest posteriorly; this was approx. 3 cm in diameter with ill-defined margins; the overlying skin was reddened.

NF 43

II-7 was referred to the study by her GP.

II-7 - 21.1.15; 70.7 years. Retired home help; SC: IV; elementary school education, no qualifications.

3 CAL; freckling: approx. 30 freckles in left axilla only; CNF: 4; face: no; LN: not examined; GNF: 2; Ht: 146 (<3); HC: 54 (50).

II-7 was a spinster who had not been formally diagnosed until the age of 67 years when she had seen a dermatologist. She complained of severe pruritis from her neurofibromas and was extremely upset by her appearance. No NF-1 complications.

Classified as new mutation.

I-1 - 28.5.1874, died 1948 aged 74 years. Died suddenly at home, cause unknown, no records available. Reported normal.

I-2 - Born 1879, died 1956 aged 77 years.

Died from carcinoma of the throat, no records available. Reported normal.

Siblings of II-7 - II-7 reported she was the only affected family member.

II-1 had died of a myocardial infarction, age uncertain; II-2 died at 81 years, cause unknown; II-5 had died of a cerebrovascular accident at 39 years. The remaining siblings of II-7 and the members of generation III were reported to have no health problems.

NF 44

IV-1 was referred to the study by his GP.

IV-1 - 3.11.73, 11.9 years; attending comprehensive school, no particular problems; SC: IIIM (father a joiner).

7 CAL; freckling: none; CNF: 1; face: no; LN: present, not counted; GNF: 4; Ht: 135 (3); HC: 56 (97).

The diagnosis of NF-1 was made at 8 months when he presented with infantile spasms and was noted to have multiple CAL spots. EEG showed hypsarrhythmia. He was treated with ACTH with resolution of the attacks and no secondary developmental problems.

At the age of 4 years he was noted to have problems with vision and at ophthalmological assessment he was found to have decreased vision in the right eye with optic atrophy. There was no proptosis and X-rays of the optic foramina were normal. Diagnosis of optic atrophy of unknown aetiology was made. When he was assessed for the study, vision in his right eye had decreased from 2/60 to counting fingers at 4 feet. Vision in his left eye was normal. On contacting the ophthalmologist it became clear he was unaware of the diagnosis of NF-1. On re-assessment his optic foramina were still normal on X-ray but CT scan showed enlargement of the right optic nerve within the orbit and no intracranial extension; the appearance was consistent with an optic nerve glioma.

IV-1 had also had a swelling over the left temple at 4 years of age. Excision biopsy in 1983 showed this to be a plexiform

neurofibroma. On examination for the study, a 2 cm diameter diffuse swelling persisted.

I-1 - Born 1872, died aged 73 years in 1955.

Reported unaffected, died from carcinoma of the breast.

I-2 - Date of birth unknown, died at 92 years. Reported unaffected, died from an aortic aneurysm.

II-1 - 16.12.16; died 1971 aged 64 years.

Reported by daughter to be first case of NF-1 in family. No known complications until presented on day prior to death with right hypochondrial pain and increasing dyspnoea. Her condition rapidly deteriorated with enormous swings of blood pressure and she died 24 hours later. Autopsy showed acute left ventricular failure and haemorrhage into a phaeochromocytoma on the right adrenal.

Classified as a new mutation but insufficient detail on parents' and siblings' birth dates to use in analysis.

Siblings of II-1 - II-2,3, and 5 had died from myocardial infarction, II-4 suffered from angina. None were reported to have NF-1.

III-1 - 8.6.40, 45.3 years; part-time cleaner; SC: V; secondary modern school education, no qualifications.

5 CAL; freckling: left axillae and submammary regions; CNF: 3; face: no; LN: not examined; GNF: 1; Ht: 151 (3); HC: 55 (75).

III-1 was first diagnosed when her son was an infant although she had no idea about the possible implications of the diagnosis. She had no NF-1 complications.

III-2 - Reported normal. Lived outside the study area.

NF 45

II-3 was referred to the study by his GP.

II-3 - 25.3.62, 23.4 years. Factory labourer; SC: V; comprehensive

school education; obtained 2 CSEs.

9 CAL; freckling: axillae and groins; CNF: 3; face: no; LN: not examined; GNF: 1; HT: 166 (10); HC: 58 (97)

The diagnosis of NF-1 was made at 6 years of age when he presented with short stature and multiple CAL spots. He was also noted to have a right 6th nerve palsy and mild conductive deafness on the left, the aetiology of both were unknown and had not changed since early childhood.

He had regular paediatric follow-up during childhood and had 2 cutaneous lesions removed, a neurofibroma at 16 years from the abdominal wall and a probable angio-pericycystic hamartoma from the left ankle at 22 years.

Classified as new mutation.

I-1 - 10.7.31, 54.2 years. Steelworker; SC: IV; secondary modern school education; no qualifications.

Normal cutaneous examination.

I-2 - 19.8.35, 50 years; part-time escort; SC: IV; secondary modern school education, no qualifications.

Normal cutaneous examination.

Siblings of II-3 - Reported normal by parents.

NF 46

II-5 was ascertained via hospital records and referred by her consultant plastic surgeon and GP with her son III-3.

II-5 - 15.2.28, 57.2 years. Housewife (former shop assistant); SC: IV; secondary modern school education, no qualifications.

6 CAL; freckling: axillae; CNF: 5; face: yes; LN: not examined; GNF: 3; Ht: 151 (3); HC: 57.5 (>97).

II-5 had been diagnosed at 20 years of age and been told the name of the disease but no other information. She was extremely disturbed by her appearance and had had numerous plastic surgery procedures for removal of neurofibromas. She had hypertension which was well

controlled on beta blockers; urinary VMAs at the time of the study were normal.

Classified as new mutation.

III-3 - 12.3.63, 22.2 years; unemployed (formerly on YTS Scheme); SC: IV; comprehensive school education, attained GCE 'O' level passes. 10 CAL; freckling: axillae; CNF: 3; face: no; LN: not examined; GNF: 1; Ht: 162.5 (3); HC: 57 (90)

II-3 had no complications of NF-1 and had not had regular review for the disease. He had several neurofibromas removed for cosmetic reasons, largely at his mother's insistence.

I-1 - Reported unaffected. Died of carcinoma of the lung.

I-2 - Reported unaffected. Died of Parkinsons's disease at 78 years.

Siblings of II-5 - II-5 said she was the only affected member of her sibship. II-2 had died from a brain haemorrhage and II-3 from carcinoma of the lung.

III-1 and III-2 - Dates of birth 16.12.61 and 21.2.59 respectively. II-5 did not want her other 2 children (III-1 and III-2-) to be seen. Neither they nor their offspring were reported to have any health problems.

NF 47

II-9 was referred to the study by his GP.

II-9 - 7.6.18, 67.2 years. Retired miner; SC: IIIM; elementary school education, no qualifications.

4 CAL; freckling: none; CNF: 5; face: yes; LN: not examined; GNF: 3; Ht: 157 (<3); HC: 58 (97)

II-9 was unsure when he had first been told of his diagnosis and was unaware of possible complications or its inheritance. He had well controlled essential hypertension and investigation had excluded a phaeochromocytoma when he had first presented in 1974. He had had

several neurofibromas removed for cosmetic reasons.

Classified as new mutation.

I-1 - Born 1876, died 1954 aged 78 years, cause unknown. Reported unaffected.

I-2 - 29.5.1877, died 1954 aged 77 years, from pneumonia. Reported unaffected.

Siblings of II-9 - II-9 reported he was the only affected member of his sibship, 8/9 of whom had died from the following:

II-1 - 24.10.97, died 79 years: pneumoconiosis

II-2 - 2.10.99, died 50 years: bronchitis

II-3 - 6.6.01, died 63 years: myocardial infarction

II-5 - Born 1905, died 37 years: cause unknown

II-6 - 11.3.08, died 65 years: pneumoconiosis

II-7 - 4.3.12, died 66 years: CVA

II-8 - Born 1916, died 1 year: cause unknown.

III-1 - 7.12.52; 33 years.

8 CAL; freckling: none; CNF: 2; face: no; LN: 0; GNF: 4; Ht: 151 (3);
HC: 54 (50)

III-1 lived outside the study area. Neither she nor her parents were aware she had NF-1 until seen for the study. At the age of 3 years she had presented with proptosis and papilloedema of the right eye. Investigation had showed enlargement of the right optic foramina and at craniotomy a right optic nerve glioma was removed. This extended back towards the chiasm and she had an enucleation with removal of the right optic nerve. She had no further problems and vision in her left eye was normal at the time of assessment. Her neurosurgical notes do not comment on CAL spots nor of her father having NF-1.

NF 48

II-5 was referred to the study by her neurosurgeon

II-5 - 23.11.62, 22.2 years. On Youth Training Scheme; SC: V; remedial class education, no qualifications; formal IQ assessment in 1984 showed IQ = 82.

8 CAL; freckling: axillae; CNF: 4; face: yes; LN: 18; GNF: 4; Ht: 158 (25); HC: 61.5 cm (centile inappropriate for analysis).

Had delayed milestones as a child and had required speech therapy from 3 years of age. At 7 years she developed ataxia and increasing headaches, examination showed bilateral papilloedema with a spastic paraparesis. Skull x-ray showed signs of chronic raised intracranial pressure and a ventriculogram hydrocephalus secondary to aqueduct stenosis. A shunt was inserted between the right lateral ventricle and upper cervical spine with satisfactory resolution of symptoms. In 1981 a ventriculo-atrial shunt had been inserted but the indications of this were unclear from the notes.

In 1984 (aged 21 years) she presented with increasing difficulty in walking; examination showed recurrence of a spastic paraparesis, in addition her blood pressure was persistently elevated.

Investigation of her hypertension excluded a pheochromocytoma but isotope scan showed delayed excretion from the left kidney and arteriogram showed a renal artery stenosis. Her blood pressure was controlled medically during neurosurgical investigation which showed that her shunt was not working and this again was revised. Spinal CT and a myelogram also showed a dumbbell neurofibroma at T3 which was subsequently removed. Following recovery from this, a right renal angioplasty had been performed. At the time of assessment for the study her symptoms and signs were slowly resolving, although she had required catheterisation 2 weeks after her spinal operation and this had not been able to be removed. Blood pressure was being controlled on decreasing doses of acebutolol and captopril. Classified as new mutation.

I-1 - 7.2.17; 68.8 years. Retired labourer; SC: V; elementary school education; no qualifications.

No abnormalities on cutaneous or slit lamp examination.

I-2 - 13.3.20; 65.8 years. Housewife; SC: V; elementary school education; no qualifications. No abnormality on cutaneous or slit lamp examination.

II-1 - 24.12.42; died 1982 aged 40 years from aspiration pneumonia. She had been an alcoholic. No features of NF-1 reported.

II-2 - 16.9.45. II-2 and her children were reported normal.

II-3 - 7.11.48; 37.1 years.

Normal cutaneous examination. Children reported unaffected.

II-4 - 12.9.59. Reported normal.

NF 49

This family were ascertained by III-4 and III-5, who had previously been referred to the genetics clinic for diagnostic clarification, and III-8 who was ascertained via hospital records.

All the members of NF 49 lived within the study area except III-9 and his family who had emigrated to Australia. Although few of the family had sought medical advice about NF-1 they were aware of the diagnosis and its genetic implications, although not of its possible complications.

The family were used in the linkage studies.

III-4 - 25.2.71; 13.2 years. SC: IIIM; comprehensive school education.

4 CAL >1.5 cm; 6 CAL >0.5 cm; freckling: none; CNF: 1; LN: 0; GNF: 1; Ht: not appropriate; HC: 57.5 (>97).

Multiple CAL spots had been noted from infancy and as she grew short stature was also obvious with an initial diagnosis of achondroplasia. In 1975 she was referred to the genetics department for evaluation. In addition to CAL spots she had limitation of extension of the elbows, moderate joint laxity elsewhere, and short broad hands and feet. Skeletal survey showed the changes of multiple epiphyseal dysplasia (MED). Her mother was found to be similarly affected, she reported her father had the same problem.

III-5 - 27.12.73; 10.4 years. SC: IIIM; no problems during primary school education.

7 CAL; freckling: axillae: CNF: 1; face: no; LN: one on left iris; GNF: 1; Ht: not appropriate; HC: 55 (>97).

Multiple CAL spots had been present from infancy. At 6 months she had also developed small yellow raised areas on her face and skin. On dermatological review NF-1 was diagnosed and biopsy of one of these lesions showed that they were juvenile xanthogranulomata. In 1977 she was referred to the genetics department for evaluation. Examination showed she had inherited both NF-1 and MED.

III-8 - 15.8.69; 14.8 years. SC: IIIM; comprehensive school education.

17 CAL; freckling: axillae and groins; CNF: 2; face: no; LN: present, unable to count; GNF: 1; Ht: 150.5 (<3); HC: 58 (>97).

CAL spots were present from infancy but III-8 had not been reviewed in hospital until he presented with pneumonia in 1980. Examination and investigation showed signs of a left lower lobe pneumonia and in addition the murmur of a patent ductus arteriosus (PDA) with a collapsing pulse. He recovered satisfactorily from his pneumonia but subsequently became dyspnoeic on exertion and the PDA was ligated in 1981 with no problems thereafter.

I-1 - 18.10.11; 72.6 years. Housewife; SC: IIIM (deceased spouse had been a miner); secondary modern school education, no qualifications.

2 CAL; freckling: axillae; CNF: 4; face: yes; LN: 29; GNF: 2; Ht: 157.4 (25); HC: 57 (>97).

The diagnosis of NF-1 had been made at 50 years when she asked for some neurofibromas to be removed for cosmetic reasons. No NF-1 complications.

She had recurrent iron deficiency anaemia, barium studies had shown no obvious source of bleeding in the gastrointestinal tract.

I-1 had been adopted and therefore the mode of inheritance of NF-1 was unknown.

II-1 - 17.3.42; 42.2 years. Control crossing attendant; SC: IV; remedial class education, no qualifications.

4 CAL; freckling: axillae; CNF: 4; face: no; LN: 46; GNF: 2; Ht: 147.5 (<3); HC: 58.5 (>97).

II-1 had had some neurofibromas removed for cosmetic reasons and would not go swimming because of her appearance.

II-2 - 16.4.43; 41.1 years. Bench viewer in car component factory; SC: IIIM; remedial class education, no qualifications.

7 CAL; freckling: axillae, groins, over trunk; CNF: 4; face: no; LN: 28; GNF: 2; Ht: 150 (<3); HC: 58 (>97).

Until he attended the genetics clinic with his daughters, he had been unaware that NF-1 had been anything other than a "family trait".

II-3 - 21.11.44; 39.6 years. Housewife; SC: IIIM; secondary modern school education, no qualifications.

11 CAL; freckling: axillae; CNF: 4; face: no; LN: 50; GNF: 2; Ht: 151 (3); HC: 55 (75).

Had a neurofibroma removed for diagnostic purposes in her 20s. Apart from anxiety about her appearance, no NF-1 related problems.

II-4 - 7.12.45; 38.5 years. Maintenance technician with Gas Board; SC: IIIM; secondary modern school education, no qualifications.

No abnormalities on examination of skin or iris. Ht: 173 (50); HC: 58 (>97).

II-5 - 24.11.46; 37.5 years. Unemployed component fitter; secondary modern school education, no qualifications.

No abnormalities on examination of skin or iris. Ht: 169 (25); HC: 56.5 (90).

II-6 - 4.11.48; 35.6 years. Ran a garage with her husband; SC: II; secondary modern school education, no qualifications.

Normal examination of skin and iris. Ht: 165 (50); HC: 54.5 (50).

II-7 - 13.10.49; 34.7 years. Greengrocer; SC: II; secondary modern school education, attained several CSEs.

18 CAL; freckling: axillae, groins, submammary regions and over trunk; CNF: 3; face: no; LN: 64; GNF: 1; Ht: 161 (50); HC: 54.5 (75)

Apart from removal of a few neurofibromas for cosmetic reasons, no NF-1 related problems.

II-8 - 23.10.50; 33.7 years. Maintenance electrician; SC: IIIM; secondary modern school education, attained 5 CSEs.
No abnormalities on examination of skin or iris. Ht: 173 (50); HC: 57 (90).

II-9 - 12.7.52. Lives in Australia. Reported affected by mother.

II-10 - 5.3.54; 30.3 years. Upholsterer; SC: IIIM; secondary modern school education, no qualifications.
No abnormality on examination of skin or iris. Ht: 172.5 (50); HC: 59 (>97).

III-1 - 21.6.66; 18 years. On YTS; SC: IV; remedial class education, attained 1 CSE pass.
6 CAL; freckling: axillae; CNF: 2; face: no; GNF: 2; LN: 41; Ht: 166 (10); HC: 58 (>97).

III-2 - 29.1.68; 16.4 years. SC: IV; attending remedial class at comprehensive school.
11 CAL; freckling: axillae and groins; CNF: 1; face: no; GNF: 2; LN: 22; Ht: 161 (<3); HC: 58 (>97).

III-3 - 8.6.71; 13 years. SC: IV; schoolgirl, attending comprehensive school.
11 CAL; freckling: axillae; CNF: 1; face: no; LN: 34; GNF: 1; Ht: 143 (3); HC: 56 (97).

The mother of III 1-3 realised the implications of CAL spots but none of them had ever been formally reviewed nor had had any NF-1 complications.

III-6 - 1.5.66; 18.4 years. SC: IIIM; secondary school education, attained 3 CSE passes.
Normal examination of skin and iris. Ht: 187 (97); HC: 60.5 (>97).

III-7 - 27.1.68; 16.4 years. On retail distribution course at College of Further Education; SC: IIIM; comprehensive school education, needed extra help with reading, attained 3 CSE passes.
20 CAL; freckling: axillae and groins; CNF: 2; face: no; GNF: 2; Ht:

165.5 (10); HC: 57 (90).

No NF-1 related problems but investigation of intermittent jaundice at the age of 16 years had shown a raised unconjugated bilirubin and no other abnormalities. The diagnosis of Gilbert's disease had been made.

III-9 - 3.2.78; 6.4 years. SC: IIIN; schoolgirl, no problems at primary school.

11 CAL all >1.5cm; freckling: none; CNF: 1; face: no; LN: 10; GNF: 1; Ht: 105.5 (3); HC: 52 (75).

Mother aware of implications of CAL spots, no formal review for NF-1.

III-10 - 30.6.81, examined at 2.9 and 4.5 years; SC: IIIN; attending primary school with no problems.

No abnormality on cutaneous and slit lamp examination. Ht: 100 (>97); HC: 53 (>97).

III-11-13 - Family uncertain regarding their disease status.

NF 50

II-1 was referred to the study by her GP.

II-1 - 3.12.71; 14 years. SC: IV; schoolgirl receiving home tuition because of heart problem, experiencing learning difficulties particularly with writing, and had poor concentration.

10 CAL >1.5 cm; freckling: none; CNF: 1; face: no; LN: 0; GNF: 2; Ht: 157 (excluded from analysis as scoliosis); HC: 56 (97)

II-1 had been diagnosed by a paediatrician at 4.5 years of age on the basis of multiple CAL spots and had been under regular review from that time. She had three main problems which were under review:

(1) Hypertrophic obstructive cardiomyopathy (HOCM)

An ejection systolic murmur was noted from early childhood but as she was asymptomatic and ECG and chest x-ray were normal it was presumed innocent. In 1982 she complained of dyspnoea on exertion and was re-investigated. ECG and chest x-ray were again normal but an

echocardiogram showed a thickened intraventricular septum. Angiography showed a subvalvular gradient of 85 mmHg and virtual obliteration of the left ventricular cavity during systole. Diagnosis of HOCM was made. She was commenced on propranolol and her condition had not deteriorated at the time of the study. Her parents were extremely anxious about her heart and insisted on home tuition against medical advice. Subsequent echocardiography had not shown the severe changes seen on angiography and when assessed for the study she was awaiting re-investigation.

(2) Right thoracolumbar scoliosis

II-1 was under orthopaedic review for minor scoliosis first noted at 10 years. There was no underlying vertebral abnormality and the curve had not progressed.

(3) Possible epileptic seizures

II-1 had one febrile convulsion at 3 years of age. From around the age of 10 years she began to have episodes of mumbling with thrashing of her limbs during sleep and of sleepwalking on other occasions. She had never been incontinent during an attack and had no other ill effects from them. An EEG in 1983 showed medium to high voltage sharp and slow wave activity appearing at 4-6 cps in all areas. Despite this, she had not been treated as it was felt the history was uncertain. She was not classified as having epilepsy in the complication studies.

On examination for the study, the only other abnormality noted was a small plexiform neurofibroma on the left lower abdomen with overlying patchy pigmentation and reddened skin.

Classified as a new mutation.

I-1 - 21.12.42; 42.9 years. Unemployed storeman; SC: IV; secondary modern school education, no qualifications.
No abnormality on examination of skin or iris.

I-2 - 17.4.47; 38.6 years. Housewife; grammar school education; attained GCE 'O' level passes.

On examination 1 CAL spot was noted and slit lamp examination was normal. Classified normal.

II-2 - 4.5.78. Reported unaffected by parents.

NF 51

II-2 was referred to the study by her ophthalmologist.

II-2 - 15.10.80; 5.1 years. SC: IV; schoolgirl, no developmental delay, no obvious problems since starting primary school.

6 CAL; freckling: none; CNF: 1; face: yes, secondary to plexiform neurofibroma; LN: 5; GNF: 4; Ht: 103 (25); HC: 51 (excluded from analysis because of facial lesion).

II-2 had a sticky right eye from birth which did not fully resolve with antibiotic ointment and at 6 weeks frank swelling of the right periorbital region was noted. On paediatric referral CAL spots were noted and skull x-ray showed enlargement of the right orbit and superior orbital fissure; biopsy of the lesion showed a plexiform neurofibroma. The lesion gradually grew in size producing proptosis and closure of the right eyelid. By 4 years she had no useful vision in the right eye and swelling of the right cheek with overgrowth of the maxilla. The superior orbital fissure had widened further and a series of plastic surgery procedures were undertaken: bone grafting and enlargement of the right orbit, debulking of the facial lesions. At surgery most of her facial muscles found to be replaced by neurofibromatous tissue.

On examination for the study, these procedures had obviously greatly improved her appearance; she and her family were extremely well adjusted to her problems.

Classified as a new mutation.

I-1 - 6.10.53; 32.1 years. Stores assistant; SC: IV; comprehensive school education; attained several CSE passes.

1 CAL spot on examination, classified normal.

I-2 - 12.2.56; 29.8 years. Housewife; SC: IV; comprehensive school education, attained several CSE passes.

Normal examination of skin and iris.

II-1 - 8.2.79; 6.8 years. Schoolgirl, no developmental problems.
1 CAL spot on examination, classified normal.

NF 52

II-2 was ascertained via hospital records.

II-2 - 13.6.55; 30.4 years. Housewife (former shop assistant); SC: IV; secondary modern school education, no qualifications.
9 CAL; freckling: axillae and groins; CNF: 4; face: no; LN: 5; GNF: 2; Ht: 153 (10); HC: 58 (>97).

Multiple CAL spots had been present from infancy. The diagnosis of NF-1 had been made at 7 years when she began to develop neurofibromas. She had several admissions from the age of 20 years from the removal of cutaneous neurofibromas for cosmetic reasons. She had been myopic from childhood; on examination for the study her vision was 6/36 in the right eye and 6/18 in the left, both corrected to 6/9.

She was unaware of the genetic implications of NF-1 or of its complications.

Classified as a new mutation.

I-1 - 11.10.27; 58.1 years.

No abnormalities on examination of skin or iris.

I-2 - 24.12.32; 52.9 years.

No abnormality on examination of skin or iris.

II-1 - 5.2.54. Reported normal by parents.

II-3 - 14.1.59. Reported normal by parents.

III-1 - 12.9.83; 2.2 years. Under paediatrician for possible delay in milestones. Had not walked until 18 months, only 2 words at time of study assessment.

No abnormalities on cutaneous examination.

IV-3 was ascertained via hospital records. As these stated his mother was affected, the family were contacted through their GP.

IV-3 - 11.9.74; died 22.11.76, aged 2.3 years.

Multiple CAL spots had been noticed by the parents from 2 months of age. His development was normal until September 1976 when he was admitted with urinary retention. After initial catheterisation had been able to micturate satisfactorily and was discharged. Six weeks later the problem recurred and a firm pelvic mass was noted on examination. At laparotomy a large lobulated mass arising from the bladder and rectum was found which could only be partially removed. Histology was of a malignant rhabdomyosarcoma. He was commenced on chemotherapy and his urinary obstruction was temporarily relieved; when it recurred his blood urea began to rise and he died only 4 weeks after the diagnosis had been made.

I-1 - Reported to have had multiple cutaneous "lumps". No other details available. Presumed affected.

II-1 - No details available.

II-2 - Died 1951, age uncertain. Reported by II-1 to have had scoliosis and to have died of chest problems. No other details available. Presumed affected.

III-1 - Born 1938, died within hours of birth, cause unknown.

III-2 - 4.7.40; 45.4 years. Housewife (former telephonist, husband signalman); SC: IIIM; secondary modern school education, no qualifications.

9 CAL; freckling: axillae and groins; CNF: 4; face: no; LN: 2; GNF: 3; Ht: 146 (excluded from analysis: pseudoarthrosis); HC: 54.5 (50).

III-2 had been born with a fractured right tibia and fibula, the fibula was rudimentary with approx. 5 cm of the lower third missing. No mention of NF-1 was found in her orthopaedic notes. Her parents had refused orthopaedic advice to have an amputation and she managed

with calipers until the age of 39 years when she decided to have a below knee amputation. At the time of assessment for study, she was fully mobile with an artificial limb.

III-2 had several neurofibromas removed for cosmetic reasons. She had not been told that NF-1 was related to her son's death or her own orthopaedic problem.

III-3 - 13.1.46. III-3 and his children were reported normal, they lived outside the study area.

IV-1 - born 17.6.71 at 31 weeks gestation. Died at 13 weeks from respiratory problems.

IV-2 - 24.11.72; 13 years. Schoolgirl, attending comprehensive school.

No abnormalities on examination of skin or iris.

NF 54

The index case was ascertained through hospital records. She had been adopted at 8 weeks and no precedent family history was available.

Index case - 29.4.78; 7.6 years. Schoolgirl, attending primary school with remedial class help for reading and writing.

13 CAL >1.5 cm, 6 CAL >0.5 cm; freckling: no; CNF: 1; face: no; LN: 1; GNF: 2; Ht: 117 (10); HC: 51 (25).

Her adoptive parents had noted CAL spots at the time of her adoption. Diagnosis of probable NF-1 was made when she had an isolated febrile convulsion at 18 months and multiple CAL spots were noted.

After starting school she had a lot of problems, with her teacher thinking she was lazy. This led to an assessment by an educational psychologist at 7 years 3 months which showed a verbal IQ of 106, performance IQ of 70 and full-scale IQ of 87. She was able to read appropriately for her age but had major difficulty in copying patterns and shapes. Classified as having learning difficulties.

On examination she had a plexiform neurofibroma, approx. 6 cm in

diameter in her left lower thoracic region posteriorly, it was covered by an area of hypertrichosis.

NF 55

II-3 was referred to the study by his GP.

II-3 - 4.5.24; 61.6 years. Retired miner; SC: IIIM; elementary school education, no qualifications.

8 CAL; freckling: none; CNF: 5; face: yes; LN: 15; GNF: 2; Ht: 171 (25); HC: 57 (90).

II-3 was unaware of the name of his disease and did not understand its nature until the time of the study. He had a few neurofibromas removed for cosmetic reasons; no other problems.

On examination he had a plexiform neurofibroma approx. 7 cm in diameter on the posterior aspect of his left arm.

Classified as new mutation.

I-1 - 8.6.00. Died 1954 aged 54 years from chronic bronchitis. Reported unaffected.

I-2 - 25.7.95. Died 1964 aged 69 years from carcinoma of breast. Reported unaffected.

Siblings of II-3 - The siblings of II-3 and their children were reported to be in good health and to have no NF-1 stigmata.

NF 56

IV-3 was referred to the study by her plastic surgeon.

IV-3 - 8.4.82; 3.6 years; normal milestones to date.

3 CAL >1.5 cm; 5 >0.5 cm; freckling: none; CNF: 1; face: yes (plexiform neurofibroma); LN: unable to examine; GNF: 4; Ht: 94 (25); HC: 52.5 (excluded from analysis, facial plexiform neurofibroma).

IV-3 was a brow presentation and at birth had generalised facial

swelling, this resolved apart from an area on her left cheek which persisted and later increased in size. On paediatric referral at one year a diffuse swelling extending from the upper cheek to lower jaw was noted. This was biopsied and histology was of a plexiform neurofibroma.

At the age of 3 years she had a more extensive plastic surgery procedure and the lesion was found to involve the greater auricular, trigeminal, facial, hypoglossal and glossopharyngeal nerves, and the upper cervical plexus.

At the time of study assessment, residual swelling was noted on the cheek and jaw area with no involvement of the eye. The mother of IV-3 was unwilling to accept that the problem was related to NF-1.

I-1 - Reported affected by II-3. Died in her 70s, cause unknown. No prior family history available.

II-1 - 11.8.24; 61.3 years. Housewife; SC: V.

II-1 was unkeen to have a full assessment; brief examination of her abdomen showed several CAL spots and multiple neurofibromas. Her only reported medical problem was long-standing bilateral deafness with tinnitus but no ataxia. Her ENT showed this was sensorineural deafness. Examination of her cranial nerves at the time of study showed no other abnormalities. She was unwilling to have further investigations of her deafness.

Siblings of II-1 - III-3 was unable to supply any details about her maternal aunts and uncles, only one of whom was said to live in the study area.

III-1 - 6.6.50. Reported normal; family unwilling for him to be contacted.

III-2 - 11.5.57. Reported normal; family unwilling for her to be contacted. Under hospital review for psoriasis. Notes do not mention any features of NF-1.

The children of III-1 and III-2 were reported normal.

III-3 - 29.11.51; 34 years. Housewife, previous factory worker; SC: V; comprehensive school education, no qualifications.

Cutaneous examination incomplete: 5 CAL seen above waist, no freckling in axillae or submammary regions; CNF: 4; face: no; LN: 5; GNF: 2; Ht: 160 (25); HC: 57 (97).

Diagnosis of NF-1 had been made at 19 years by a dermatologist although III-3 did not really understand the natural history or genetic implications of the disease.

On the upper part of her back she had a large area of CAL pigmentation in the centre of which was a plexiform neurofibroma 16 cm in diameter.

IV-1 - 26.8.71; 14.3 years. Attending remedial class at comprehensive school

IV-1 would only allow the top half of his body to be examined; there were no abnormalities.

IV-2 - 3.9.78; 7.2 years. Schoolboy, no problems to date.
Normal cutaneous examination.

IV-4 - 20.2.84; 1.8 years. Normal development at time of study.
This child would only co-operate with limited examination, 2 CAL spots were seen on his back; classified as diagnosis equivocal.

NF 57

II-1 was referred to the study by his GP and consultant surgeon. Unfortunately at the time he was contacted for the study he was undergoing treatment for carcinoma of the penis and was unwilling to be seen. The diagnosis was in no doubt from his hospital records which described multiple cutaneous neurofibromas and CAL spots.

Past medical history from hospital records -

1974 - presented with rapidly increasing swelling on right side of chest and clinically shocked. Found to have haemorrhaged into a neurofibroma from a subcostal artery. After resuscitation, lesion was removed with no complications.

Had a further neurofibroma removed from the right thigh in the same year.

1981 - Shadow on left lung field found on routine chest x-ray. Thoracotomy for excision of benign neurofibroma in 1982.

1985 - presented with indurated area on foreskin. Biopsy showed a low grade verrucous squamous cell carcinoma of the penis. Subsequent partial amputation of penis.

Family history - Family of II-2 lived in Yorkshire, his father, who was deceased, was said to have been affected as was one of his 6 brothers, there are no other family details recorded.

NF 58

II-5 was ascertained by hospital records.

II-5 - 2.3.24; 61.8 years. Spinster who had never worked; SC: IV (father had been a miner); elementary school education, described as needing extra help, classified as remedial class education.

CAL spots not counted as incomplete examination; freckling: axillae; CNF: 4; face: yes; LN: not examined; GNF: 4; Ht: 150 (3); HC: 57 (>97).

II-5 had heard the name neurofibromatosis mentioned but understood very little about the disease. She had had only one neurofibroma removed for cosmetic reasons. A routine chest x-ray in 1982 had shown a mass at the thoracic inlet, isotope scan showed a 6 cm mass with a whorled appearance and well-defined edge which was thought to be typical of a neurofibroma; as she was asymptomatic this was not removed.

In 1983 (aged 59 years) she presented with upper abdominal pain and vomiting. A clinical diagnosis of acute cholecystitis was made. An ultrasound scan however showed a dilated extrahepatic duct but no gallstones. At laparotomy an encapsulated tumour surrounding the common bile duct orifice was found and enucleated. Initial histology was of an ampullary carcinoma. This was reviewed at the time of the study at the author's request by Professor E D Williams because of his description of the association between duodenal carcinoids and

NF-1. He found that the tumour was a somatostatin-rich duodenal carcinoid.

At the time of her study assessment the patient was asymptomatic and urinary VMAs were normal.

Classified as a new mutation.

I-1 - 3.9.88. Died 1967 aged 79 years from a CVA. Reported normal.

I-2 - Died aged approx. 70 years following a fractured neck of femur. Reported normal.

Siblings of II-5 - II-5 reported she was the only affected family member. I-1 died in childhood, cause unknown; II-2 died at 26 years in an accident and II-3 had suffered from epilepsy, dying during a seizure at 46 years.

NF 59

II-2 was ascertained via hospital records.

II-2 - 14.1.58; 27.9 years. Production line worker in chocolate factory; SC: V; comprehensive school education, learning difficulties particularly with mathematics, no qualifications.

13 CAL; freckling: axillae, groins and submammary regions; CNF: 4; face: no; LN: 9; GNF: 1; Ht: 155 (10); HC: 58 (>97).

Diagnosis of NF-1 had been made at 21 years when II-2 was referred to a plastic surgeon for removal of neurofibromas for cosmetic reasons. She had no other NF-1 related problems apart from a bony defect of the right lamboidal suture on examination. II-2 reported this had first been noted on x-ray after an accident in her teens.

Classified as new mutation. Her son had been adopted in infancy and was therefore not available for assessment.

I-1 - 23.8.27. Not in contact with family, reported unaffected by former wife.

I-2 - 12.6.29; 56.5 years. Nursing sister; SC: II; grammar school education, attained GCE 'O' level passes
No abnormality detected on examination of skin and iris.

II-1 - 3.7.55. Lived outside study area, reported unaffected by her mother.

NF 60

II-1 was ascertained through hospital records.

II-1 - 7.2.46; 39.8 years. Domestic help; SC: IV; secondary modern school education, no qualifications.

11 CAL; freckling: axillae and groins; CNF: 3; face: no; LN: 14; GNF: 2; Ht: 150 (not used in analysis - scoliosis); HC: 57 (>97).

II-1 was aware of the diagnosis of NF-1 after dermatological review at 38 years. It was however noted in her hospital records at 18 years when she had a dental surgery procedure for reduction of prognathism; there is no evidence in the notes that this problem was related to NF-1.

In 1984 (aged 38 years) she was referred to a dermatologist and found to have both NF-1 and psoriasis. Discrete upper thoracic scoliosis was also noted; x-ray showed abnormalities at T2-4 with fusion of the vertebral bodies and hypoplastic "ribbon" ribs. II-2 had been unaware of this prior to 1984 and the lesion was asymptomatic.

On assessment for the study her main complaints were of the cosmetic appearance of her neurofibromas and the intermittent intense pruritis they caused.

Classified as a new mutation.

I-1 - 12.4.14. Died 1.1.81 aged 66 years from cor pulmonale secondary to chronic bronchitis and emphysema. No features of NF-1 recorded in hospital records or reported by his wife.

I-2 - 11.2.24; 60.9 years. Home help; SC: IV; secondary modern school education, no qualifications.

No abnormalities on examination of skin or iris.

II-2 - 10.2.48. Reported unaffected. Family unkeen for him to be contacted.

III-1 - 6.11.69; 16.1 years. Schoolgirl; SC: IV (father: steel worker); attending comprehensive school, no educational problems.

No abnormalities on examination of skin or iris.

III-2 - 30.11.73; 12 years. Schoolgirl; SC: IV; attending comprehensive school, no educational problems.

12 CAL; freckling: none; CNF: 1; face: no; LN: 10; GNF: 1; Ht: 151 (50); HC: 55 (90).

III-2 had no medical problems. Her mother was aware that she had probably inherited NF-1 but this was only confirmed formally when she was assessed for the study.

NF 61

II-2 was referred to the study by her GP.

II-2 - 5.12.52; 33 years. Housewife; SC: IIIM (husband foreman in shoe factory); secondary modern school education, no qualifications.

15 CAL; freckling: none; CNF: 4; face: yes; LN: 80; GNF: 2; Ht: 154 (10); HC: 54 (50).

The diagnosis of NF-1 had been made by her GP when her numerous cutaneous neurofibromas began to appear, although her mother did report that the CAL spots had been present from one year of age.

At 24 years she had a sclerosing haemangioma removed from her nose.

On examination she had 2 plexiform neurofibromas, one 3 cm in diameter on her forehead and the other 10 cm in diameter just above her right buttock.

Classified as a new mutation.

I-1 - 26.11.23; 62.1 years. Self-employed builder; SC: IIIM; grammar school education, no qualifications.

No abnormalities on examination of skin or iris.

I-2 - 5.5.24; 61.7 years. Housewife; secondary modern school education, no qualifications.

No abnormalities on examination of skin or iris.

II-1,3,4 - 8.8.46, 8.1.58, 25.11.68 respectively.

The siblings of II-2 and the offspring of II-1 were reported to be unaffected.

III-1 - 24.6.74; 11.5 years. Schoolboy; SC: IIIM; no educational problems.

No abnormalities on examination of skin or iris.

III-2 - 29.5.82; 3.5 years. Schoolboy; SC: IIIM; no educational problems.

1 CAL spot >1.5 cm, unable to co-operate with slit lamp examination. Classified as disease status equivocal.

NF 62

III-3 was referred to the study by her GP.

III-3 - 21.10.52; 33.1 years. Unable to work; SC: IV (husband: machine attendant); special school education, moderate intellectual handicap.

10 CAL; freckling: axillae, submammary regions and groins; CNF: 3; face: no; LN: present, not counted; GNF: 3; Ht: 156 (10); HC: 53 (10).

III-3 was illiterate and had been under psychiatric care from her early 20s for an anxiety state. She had never been formally assessed for NF-1.

On examination she had a plexiform neurofibroma on her right buttock, this was approx. 8 cm in diameter with overlying skin hypertrophy.

The family history was supplied by III-1 who was unkeen that her

mother or III-2 (reported normal) should be contacted.

II-1 - 24.11.13. Died 13.11.75 aged 62 years from pulmonary embolism complicating a deep venous thrombosis. Reported to be affected by family and GP although no mention of NF-1 in hospital records. No details regarding disease status of his parents or siblings available.

IV-1 - 12.3.76. IV-1 was in foster care, outside the study area, was reported to have CAL spots although was excluded from analysis as not personally examined.

NF 63

II-2 referred himself to the study after reading an article about it in the local newspaper.

II-2 - 2.10.29; 56.3 years. Unemployed, former planning officer with Water Authority; SC: IIIM; elementary school education, no qualifications.

14 CAL; freckling: axillae and groins; CNF: 3; face: no; LN: 2; GNF: 1; Ht: 168.5 (25); HC: 55.5 (75)

II-2 had never sought medical advice about his minor skin manifestations of NF-1. No other medical problems.

Classified as new mutation.

I-1 - 16.4.1892, died 1964 aged 72 years from CVA. Reported normal.

I-2 - 18.9.1893. Reported normal. Family did not want her upset by being examined for study.

II-1 - 2.4.23. Interviewed, not examined. Reported no abnormalities. His eldest son (III-1) had died at 32 years from renal carcinoma. His other 2 children had no health problems.

III-4 - 2.6.67; 18.6 years. Student at College of Further Education (science technician course); SC: II; comprehensive school education, attained several CSE passes.

One CAL spot on examination, no neurofibromas or Lisch nodules. Classified unaffected.

III-5 - 29.10.73; 12.2 years. Schoolboy; SC: IIIM; no educational problems.

13 CAL; freckling: axillae and groins; CNF: 1; face: no; LN: 0; GNF: 1; Ht: 138 (10); HC: 55.5 (90).

No medical problems, diagnosed secondary to study.

NF 64

III-1 and III-2 were referred by their paediatrician.

III-1 - 2.8.71, 14.4 years. Schoolgirl; SC: IVM (father worked in remploy factory); attending comprehensive school, no educational problems.

19 CAL; freckling: axillae and groins; CNF: 1; face: no; LN: 9; GNF: 1; Ht: 146.5 (excluded from analysis because of possible osteogenesis imperfecta (OI)); HC: 55.5 (90).

Diagnosis of NF-1 had been made on the basis of multiple CAL spots at 6 years of age, when she was referred to a paediatrician because of asthma.

On examination for the study she had no other NF-1 problems but had blue sclerae. Her father, II-5, had OI type 1 with blue sclerae and a history of multiple fractures following minor trauma. Although III-1 had only had one fracture (in her foot when she fell off a wall) it was felt that she had inherited OI from her father and NF-1 from her mother.

III-2 - 5.2.75, 10.9 years. Schoolgirl; SC: IVM; no educational problems at primary school.

15 CAL; freckling: none; CNF: 2; face: no; LN: unable to cooperate with examination; GNF: 2; Ht: 122 (3); HC: measurement not recorded accurately.

The diagnosis of NF-1 had been made at 2 years when she had been first assessed by a paediatrician for asthma. At 6 years she had one neurofibroma removed from her right arm.

On examination for the study there were no other cutaneous

neurofibromas but she had a plexiform neurofibroma, some 4 cm in diameter, on the right of her abdomen. Her sclerae were white and she had had no fractures.

I-1 - 18.10.13; died 1976 aged 62 years, from a myocardial infarction. Reported unaffected by II-4 and no features of NF-1 recorded in hospital notes.

I-2 - 23.4.14; reported unaffected by II-4 who did not want her to be contacted for the study.

II-4 - 14.1.50; 36 years. Housewife; SC: IV; secondary modern school education, no qualifications.

9 CAL; freckling: axillae, submammary region and groins; CNF: 3; face: no; LN: 4; GNF: 1; Ht: 149 (<3); HC: 58 (>97).

II-4 viewed NF-1 as a minor skin problem and had no major medical problems. She had been on a diuretic for hypertension from her GP for one year at the time of study assessment. Her blood pressure was well controlled and urinary VMA, organised through the study, was normal on one occasion. II-4 failed to attend for hospital follow-up after her initial home assessment.

Classified as a new mutation.

Siblings of II-4 - II-4 reported she was the only affected family member in her generation. II-1 had died of a myocardial infarction at 37 years of age. II-4 did not want her siblings contacted about the study.

NF 65

The index case was ascertained through hospital records. She refused to be seen for the study and the GP could not supply an accurate family history; no pedigree therefore available.

The index case was born on 17.9.59 and NF-1 had been diagnosed at 13 years when she presented with scoliosis and multiple CAL spots; >6 spots were counted on the old clinical photos in her orthopaedic

records. She had presented with a left upper thoracic scoliosis which progressed from 20° to 30° in six months; there was no underlying vertebral abnormality. A spinal fusion of T1-7 had been carried out because of the progression, the curve was corrected to 20° and remained stable until discharge from follow-up 3 years later. Her GP reported no major medical problems from that time.

Although her orthopaedic records record no family history of NF-1, it was felt she should not be definitely counted as a new mutation.

The index case was unmarried at the time of the study.

NF 66

II-2 was referred to the study by his paediatrician.

II-2 - 30.8.67, 18.4 years. Labourer in shoe factory; SC: IV; remedial class education, no qualifications.

20 CAL; freckling: axillae and groins; CNF: 2; face: no; LN: 30; GNF: 2; Ht: 162 (3); HC: 55 (50).

The diagnosis of NF-1 had been made at 15 years when he was referred to a paediatrician for assessment of headaches and short stature. His history was of classical migraine, the attacks becoming less frequent when he stopped eating cheese. His height was below the 3rd centile on initial review, with a bone age of 12 years and a normal pituitary fossa on x-ray. Growth velocity was normal on follow-up and no further investigations were undertaken.

Classified as a new mutation.

I-1 - 20.6.38, 47.5 years. Miner; SC: IV; secondary modern school education, no qualifications.

No abnormalities on cutaneous or slit lamp examination.

I-2 - 5.10.39, 43.3 years. Home help; SC: IV; secondary modern school education, no qualifications.

1 CAL spot in right axilla; no LN or neurofibromas; classified unaffected.

II-1 - 23.6.60. Reported unaffected by parents.

II-3 - 11.1.71. Reported unaffected by parents.

NF 67

II-1 was ascertained via hospital records.

II-1 - 24.8.58; 27.4 years. Production line worker in steel works; SC: IV; comprehensive school education, no qualifications; needed extra help with reading.

12 CAL; freckling: groins; CNF: 3; face: no; LN: 0; GNF: 2; Ht: 169 (25); HC: 58.5 (>97).

The diagnosis of NF-1 had been made at 20 years of age when he had a plexiform neurofibroma partially removed from his left arm. He had a further operation for removal of a neurofibroma from his chin at 24 years but no other medical problems. He was unaware of the complications or genetic implications of NF-1.

On examination he had an ill-defined plexiform neurofibroma, approx. 8 cm in diameter, on his left arm with overlying CAL pigmentation and hypertrichosis.

Classified as a new mutation.

I-1 - 21.3.30, 55.8 years. Theatre orderly; SC: IV; grammar school education, obtained School Leaving Certificate.

No abnormality on examination of skin or iris.

I-2 - 18.7.32, 53.5 years. Nursing auxilliary; SC: II; secondary modern school education, no qualifications.

No abnormality on examination of skin or iris.

II-2 - Reported unaffected by parents.

III-1 - 30.5.83; 2.6 years. Normal developmental milestones.

4 CAL >1.5 cm, 12 CAL >0.5 cm; freckling: none; CNF: 1; face: no; LN: unable to cooperate with examination; GNF: 1; Ht: 84.5 (10); HC: 51.5 (97).

Multiple CAL spots had been noted by parents from birth; they were unaware of their implications.

On examination she had a plexiform neurofibroma on the medial aspect of the left foot, the lesion was approx. 4 cm in diameter on palpation and the left foot had been a half size bigger than the right on measurement for shoes just prior to being assessed for the study. Regular paediatric assessment suggested to the GP following review for the study.

NF 68

IV-2 was referred to the study by his former paediatrician.

IV-2 - 23.10.67, 18.2 years. Unemployed, former shelf filler in a shop; SC: IV; comprehensive school education, attained 4 CSE's (grades 4 and 5).

9 CAL; freckling: axillae and groins; CNF: 2; face: no; LN: not examined; GNF: 1; Ht: 176 (50); HC: 59 >97)

NF-1 was diagnosed at 2 years of age on the basis of multiple CAL spots. He had no medical problems but had been brought up in a children's home from 7 years of age because of parental violence.

I-1 and I-2 - This couple had been divorced and III-1 had lost touch with I-1. I-2 had died at 78 years of age, cause unknown. They were thought not to have had NF-1.

II-1 - 5.2.19. Died 1969 aged 60 years from carcinoma of the stomach. III-1 reported that her father was definitely affected; unable to trace hospital records or death certificate for further details. Excluded from study of analysis of new mutations because of uncertain family details.

III-1 - 9.8.47, 38.4 years. Cashier/clerk; SC: IIIN; grammar school education, no qualifications.

5 CAL; freckling: axillae and submammary regions; CNF: 4; face: no; LN: 60; GNF: 2; Ht: 164 (excluded from analysis: scoliosis); HC: 54.5 (50).

(50).

III-1 had been under orthopaedic review during her teens for scoliosis but no surgery had been required. No other medical problems.

On examination she had a discrete left upper thoracic scoliosis which was asymptomatic.

III-2 - Reported normal.

III-3 - Died at 5 months from spina bifida.

IV-1 - 14.6.66. Reported normal.

NF 69

The index case was referred to the study by his GP. When he was contacted about the study, the index case did not want to be seen but allowed his GP to send a report. He was born on 31.7.42, was a bachelor and his parents were said to be unaffected. The diagnosis of NF-1 had been made at the age of 18 years by a dermatologist on the basis of multiple CAL spots and neurofibromas. He had no medical problems other than bilateral Dupuytren's contracture.

Classified as a new mutation as his GP was very sure his parents were unaffected. No pedigree available.

NF 70

This family were ascertained by the British Neurofibromatosis Patients Association LINK.

II-2 - 19.3.55, 29.5 years.

10 CAL; freckling: axillae groins and trunk; CNF 3; face: normal; LN:14; Ht: 154 (10); HC: 56 (90th).

I-1 - 21.5.24, died aged 56 years from left ventricular failure due to ischaemic heart disease. Unable to trace medical records, but

family report moderate scoliosis onset 7 years of age and untreated. By the time of her death she had developed extensive dermal neurofibromas. Reported to be first case of NF-1 in family.

II-3 - 9.3.58, 26.5 years.

Examination of skin and iris normal. Ht: 186 (>97); HC: 59 (>97).

II-4 - 11.1.64, 21 years.

10 CAL; freckling: axillae, groins; CNF 4; face: normal; LN:0; Ht: 164 (<3); HC: 59 (>97).

Dermal neurofibroma removed from pressure site on neck aged 14 years. No other complications.

III-1 - 10.2.81, 3.6 years.

9 CAL > 1.5 cm, 4 > 0.5 cm; freckling:axillae; CNF 1; face:abnormal; LN:0 (portable examination); Ht: 97.5 (50); HC: 52 (75).

Bulging of left eye noted at birth, initially thought to be due to birth trauma. CAL appeared at 8 weeks and diagnosis made of NF-1 with probable plexiform neurofibroma of left orbit. This lesion had enlarged very slowly as III-1 grew but at the time of assessment he still had useful vision in the left eye although the cosmetic disfigurement was considerable. CT scans had shown no associated sphenoid wing abnormalities and surgery had not been advised whilst vision was still present unless cosmetic problems became unacceptable to the family.

NF 71

This family was ascertained when III-1 was referred to the Swansea Genetics Clinic for counselling.

III-1 - 30.3.67, 17.3 years.

16 CAL; freckling:axillae and groins; CNF 3; face: normal; LN:7; Ht: 153 (10); HC: 56 (90).

Benign neurofibroma removed from neck aged 14 years because of pain. Extremely anxious regarding NF-1.

I-1 - 30.10.08, died aged 64 years.

Family reported extensive dermal neurofibromas in adulthood; no other complications. Died within hours of myocardial infarction. Post-mortem confirmed myocardial infarction but also showed right adrenal tumour, histology not given, not known to be previously hypertensive.

II-1 - 29.6.44, 40 years.

21 CAL; freckling: axillae, groins and trunk; CNF 4; face: normal; LN:18; Ht: 145.5 (<3); HC: 55 (75).

Extremely anxious with numerous complaints of non-organic nature. Episodes of ataxia and complaints of leg weakness led to neurological investigation in 1983. Cranial CT scan was normal but a myelogram showed a small extrinsic cord lesion at L3-4. As her symptoms could not be explained by this she remains under observation.

III-2 - 21.12.68, 15.5 years.

Normal dermatological examination. Ht: 161.5 (3); HC: 53 (10).

NF 72

III-1 and III-2 were co-founders of LINK.

III-1 - 25.10.46, 38 years.

9 CAL; freckling: groins and axillae; CNF 3; face: no; LN:0; Ht: 179.5 (75); HC: 59 (>97).

III-1 was aware of his diagnosis from his early teens and had genetic counselling when first married. A small plexiform neurofibroma had been removed from his chest wall for cosmetic reasons.

I-2 - 16.8.1889, died 84 years.

Described as having extensive cutaneous neurofibromas. No medical records available but death certificate recorded cause of death as a squamous cell carcinoma of the right ear. No preceding family history available.

II-2 - 17.10.16, died aged 59 years.

Described by family as having a lot of cutaneous neurofibromas. Only known complication was of obstruction of left external auditory meatus by a cutaneous neurofibroma which required removal because of deafness. No medical records available regarding cause of death, death certificate gives caused as congenital cardiomyopathy.

II-3 - No accurate details available. Known to have been affected and died following myocardial infarction.

II-5 - 21.4.20, 64 years.

4 CAL; freckling: none; CNF:4; face: no; LN:6; Ht: 156.5 (<3); HC: 59 (>97).

On examination 5 x 12 cm plexiform neurofibroma noted over left buttock with slight skin hypertrophy. II-5 had noticed this developing over a 20-year period approximately, but it was entirely asymptomatic. Over the last 2 years had noticed deafness in the right ear; ENT assessment had shown this to be secondary to a cutaneous neurofibroma occluding the right external auditory meatus.

II-6 - Said to be affected. Family had lost touch with her and her offspring.

II-7 - Said to be unaffected.

III-3 - 21.10.49, 35 years.

Normal examination of skin and iris. Ht: 169 (90); HC: 54 (50).

III-4 - 21.3.52, aged 32 years.

9 CAL; freckling: groins and axillae; CNF:3; face: no; LN:18; Ht and HC: not appropriate.

Noted to have bowing of the right leg shortly after birth and neurofibromatosis with pseudoarthrosis of tibia and fibula diagnosed at 4 months. Treated conservatively until age 6 when had knee amputation. As long as she can remember has also had swelling over right buttock which on examination was a 25 x 25 cm plexiform neurofibroma with skin hypertrophy.

At the age of 11 she had noticed slight decrease in her vision,

more on the right. On investigation she had been found to have bilateral optic atrophy and slight enlargement of the right optic foramen. A clinical diagnosis of bilateral optic glioma was made and she was managed conservatively. Her vision remained stable however until 1979 when she noticed some decrease in vision on the right. She was fully reinvestigated: V.A. 6/36 right, 6/8 left, skull X-ray: enlarged optic foramina R=L=7mm diam.; cranial CT with orbital views and coronal sections: slight thickening of left optic nerve only; metrizamide cisternogram and carotid angiography with orbital venograms normal. It was felt that there was not enough evidence to confirm the diagnosis of bilateral optic gliomas and she has remained under observation with no further deterioration in vision.

III-6 - 5.1.51, 33.7 years.

9 CAL; freckling: axille and groins; CNF:2; face: no; LN:11; Ht: 170.5 (50); HC: 59.5 (>97).

Had one cutaneous neurofibroma removed from trunk because of severe pain on pressure. On examination noted to have one hypopigmented patch on right arm.

III-7 - 7.10.52, 32 years.

Normal examination of skin and iris. Ht: 179 (75); HC: 56.5 (90).

III-8 - 21.1.55, 29.7 years.

Normal examination of skin and iris. Ht: 175.5 (50); HC: 55.5 (75).

III-9 - 14.6.58, 26.3 years.

Normal examination of skin and iris. Ht: 153.5 (10); HC: 54.5 (50).

IV-1 - 1.4.76, 8.5 years.

5 CAL >1.5cm, 12 CAL>0.5 cm; freckling:axillae and groins; CNF:1; face: no; LN:0; Ht: 129 (50); HC: 54.5 (97).

Only problem has been with slight difficulty in reading/writing at school. Formal psychological testing age 7.5 years had showed low average ability with verbal skills being better than performance skills.

IV-2 - 19.6.79, 5.25 years.

On examination, 1 CAL >1.5 cm diameter and another less definite area on anterior chest wall. Unable to examine with portable slit lamp. Nil else.

Classified as equivocal for chromosome 19 linkage study; re-examination at 7 years showed no change, therefore subsequently classified as normal.

IV-3 - 26.3.70, 14.5 years.

Normal examination of skin and iris. Ht: 153 (3); HC: 54 (25).

IV-4 - 28.1.72, aged 12.7 years.

Normal examination of skin and iris. Ht: 150.5 (25); HC: 53 (25).

NF 73

This family was ascertained via III-6, a LINK member.

III-6 - 12.3.48, 36.5 years.

10 CAL; freckling: axillae, groins and all over trunk; CNF:3; face: no; LN: 25; Ht: 170.5 (25); HC: 63 (>97).

III-6 was diagnosed when his daughter, IV-6, presented with pseudoarthrosis. He had problems with reading and writing at school. The other problem was long-standing hearing loss in the left ear, dating from middle childhood. This was intensively investigated in 1977 when a left sensorineural deafness was demonstrated, x-rays of the internal auditory meati were normal, myodil cysternography showed a probable small lateral filling defect, possibly due to an intracanalicular acoustic neuroma. However, this investigation was repeated a year later and the examination was within normal limits. Since that time his hearing has not deteriorated and there were no other cranial nerve signs on examination; the provisional diagnosis of an acoustic neuroma seems therefore unlikely.

I-1 - Born 1887, died aged 68 years. Said to have multiple cutaneous neurofibromas in later years. Died of cancer of the bowel. No precedent family history available.

II-1 - 28.9.23, 61.2 years.

7 CAL; freckling: axillae; CNF:4; face:no; LN:0; Ht: 171 (25); HC: 61 (>97).

Neurofibroma removed from left vagal nerve aged 33 years, residual vocal chord paralysis. Investigation of abdominal pain in 1974 revealed a neurofibroma in the stomach. No active treatment, problems resolved.

II-2 - 28.12.20, 63.9 years.

10 CAL; freckling: axillae, groins; CNF:4; face: no; LN: 6; height: 173 (50); HC: 60 (>97).

II-2 had been treated for thyrotoxicosis with radioactive iodine in 1976 but never had an NF-1 complication. He complained of intermittent severe itching from his cutaneous lesions. Examination showed a plexiform neurofibroma of the right lower abdominal wall 10x10 cm diameter, slight thoracic kyphosis and pectus excavatum.

II-4 - Age unknown. Lives in Eire. Her and 13 children reported to be unaffected by family.

II-5 - 7.1.14, 70.9 years.

2 CAL; freckling: axillae; CNF: 4; face: no; LN:9; height: 158.5 (25); HC: 58 (>97).

Late onset grand mal seizures from 50 years, cause unknown, well controlled on standard therapy.

II-7 - 13.2.15, 70 years.

6 CAL; freckling: axillae and groins; CNF:4; face:no; LN: not examined; height: 158 (25); HC: 56 (90).

Had had tuberculosis of the uterus when aged 40 years. Said the family thought NF-1 was caused by her grandmother eating a lot of belly pork when pregnant!

II-8 - 16.8.19, 65.1 years.

7CAL; freckling: none; CNF: 4; face: no; LN: 5; height: 166 (10); HC: 58.5 (>97).

Apart from a partial gastrectomy for peptic ulceration, no medical problems.

III-1 - 5.7.50, 34.4 years.

Examination of skin and iris normal. Ht: 172 (97).

III-2 - 28.3.55, 29.7 years.

11 CAL; freckling: axillae; CNF: 2; face: no; LN: 10; height: not measured; HC: 57 (>97).

Had had polio at age of 2 years with residual lower limb weakness. Required a right leg caliper and elbow crutches to walk. At the age of 12 she had developed scoliosis requiring surgery; the orthopaedic surgeon felt that this was due to polio rather than NF-1. Her mobility problems persisted at the time of examination but she had had no problems related to NF-1.

III-3 - 19.3.37, 47.7 years.

5 CAL; freckling: axillae and over whole trunk; CNF: 4; face: no; LN:23; Height: 157.5 (25); HC: 56 (90).

III-5 - 17.7.43, died 31 years.

Said to have had multiple CAL spots and a few cutaneous neurofibromas prior to death from a brainstem tumour (pathology unknown). Also had a facial plexiform neurofibroma which had required multiple operations.

IV-1 - 8.4.63, 21.7 years.

6 CAL; freckling: axillae and submammary regions; CNF: 3; face: no; LN: 2; height: 160 (25); HC: 55 (75).

IV-1 had 2 small asymptomatic plexiform neurofibromas on her trunk.

IV-2 - 11.11.65, died February 1968.

Had multiple CAL spots. In June 1967 had a right nephroblastoma removed but died of multiple metastases in February 1968.

IV-3 - 11.1.68, 16.9 years.

5 CAL; freckling: axillae and groins; CNF: 3; face: no; LN: 2; height: 170 (25); HC: not appropriate.

IV-3 presented with intermittend headaches and ataxia at the age of 10 years. Investigation showed a right cerebellar mass and a grade 2 astrocytoma was completely excised. Follow-up CT scans have been

normal and he has had no residual problems. He had a benign cutaneous neurofibroma removed from his trunk at 15 years of age.

IV-4 - 25.1.70, 14.9 years.

9 CAL; freckling: unilateral axillary and bilateral in groins; CNF: 1; face: no; LN: 3; height: 158 (25); HC: 54.5 (50).

IV-4 had a small asymptomatic plexiform neurofibroma on her back, with overlying skin hypertrophy but no other problems.

IV-5 - 9.1.70, 14.7 years.

7 CAL; freckling: none; CNF: 2; face: no; LN: 5; height: 148.5 (<3); HC: 58 (>97).

IV-6 - 17.6.72, 12.2 years.




















13 CAL all >1.5 cm; freckling: groins; CNF: 1; face: no; LN: 5; height: not appropriate; HC: 54 (10).

Her parents noted curvature of the right leg when one month old. This was due to a pseudoarthrosis of the right tibia which, despite various procedures, did not unite and she had a below knee amputation at 4.7 years.

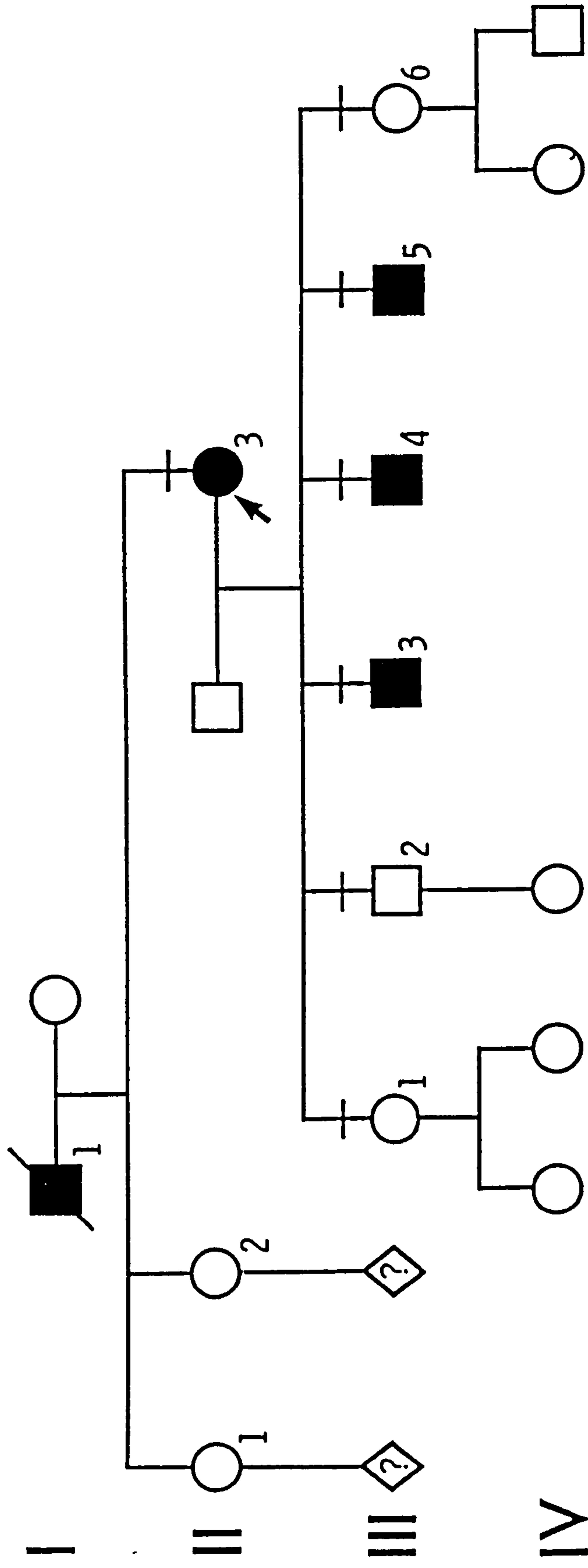
IV-7 - 17.2.79, 5.6 years.

IV-7 had 2 CAL spots >1.5 cm but no other features (slit lamp examination normal). She was classified as equivocal for the initial linkage studies but re-examination 18 months later showed no new features and she has been classified as normal from that time.

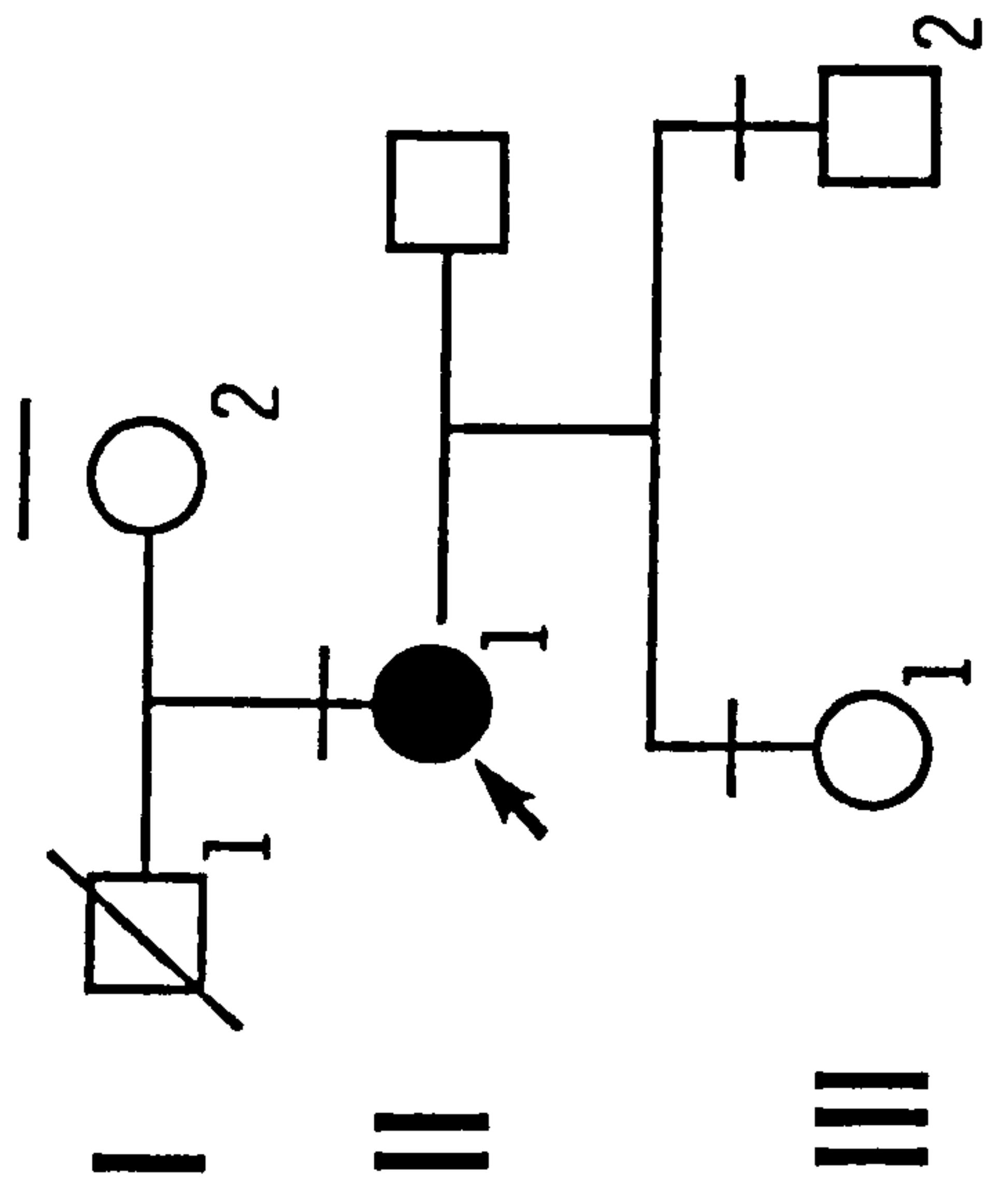
Key to Pedigrees

NF 1	Family classification number
 	Unaffected individuals (male, female respectively)
	Personally examined
 	Affected individuals
	Index case
	Diagnosis equivocal
	Sex uncertain
	Number and sex of children uncertain
 	Number of children of given sex, if > 1
	No pregnancies
	Deceased
	Spontaneous miscarriage, unless stated in text
	Dizygotic twins
	Illegitimate mating
	Details re marital status and offspring uncertain.
 	Spouses/partners of unaffected relatives have only been shown if they were married and had no children, if they had been married more than once or if they had illegitimate offspring.

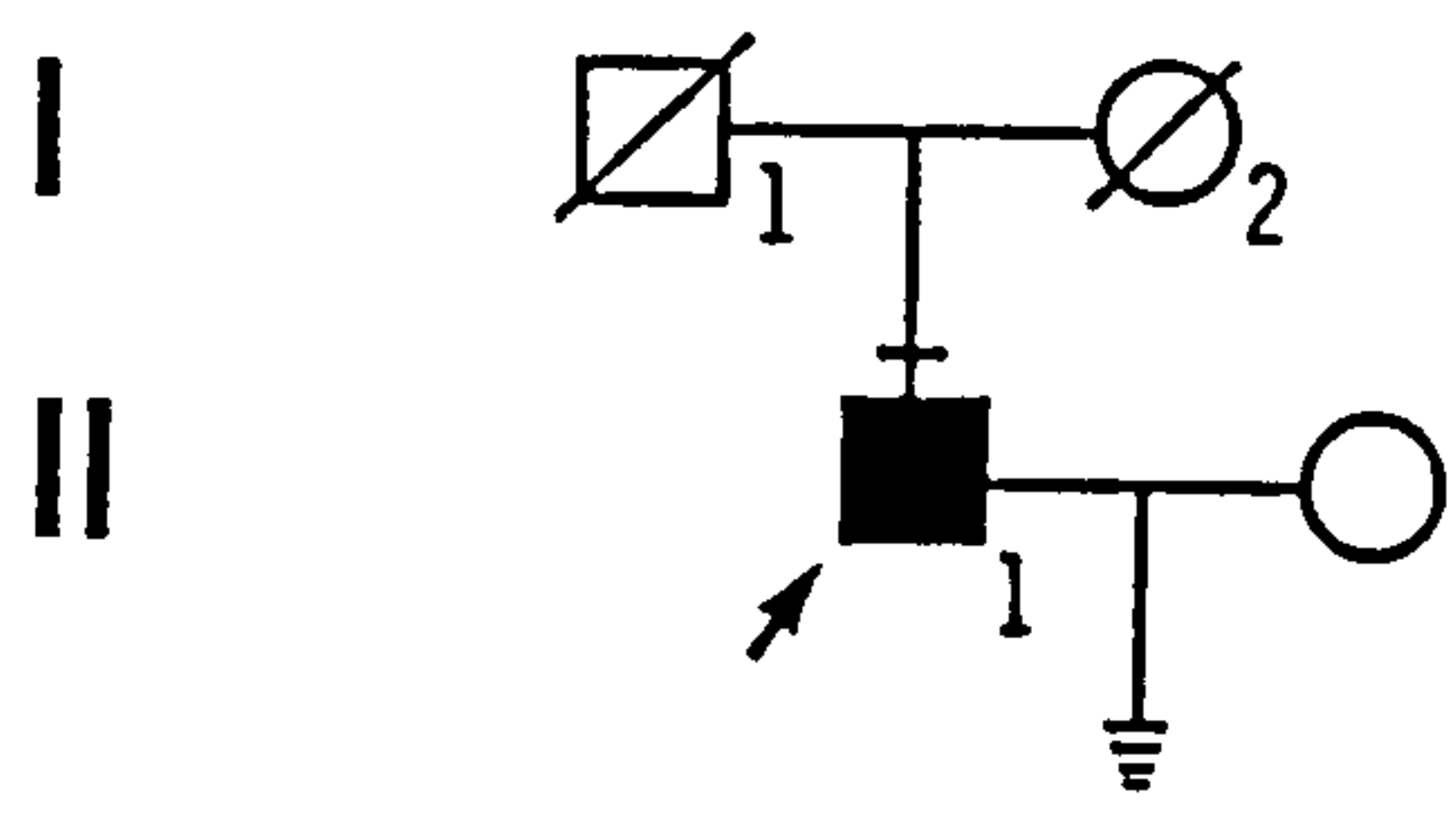
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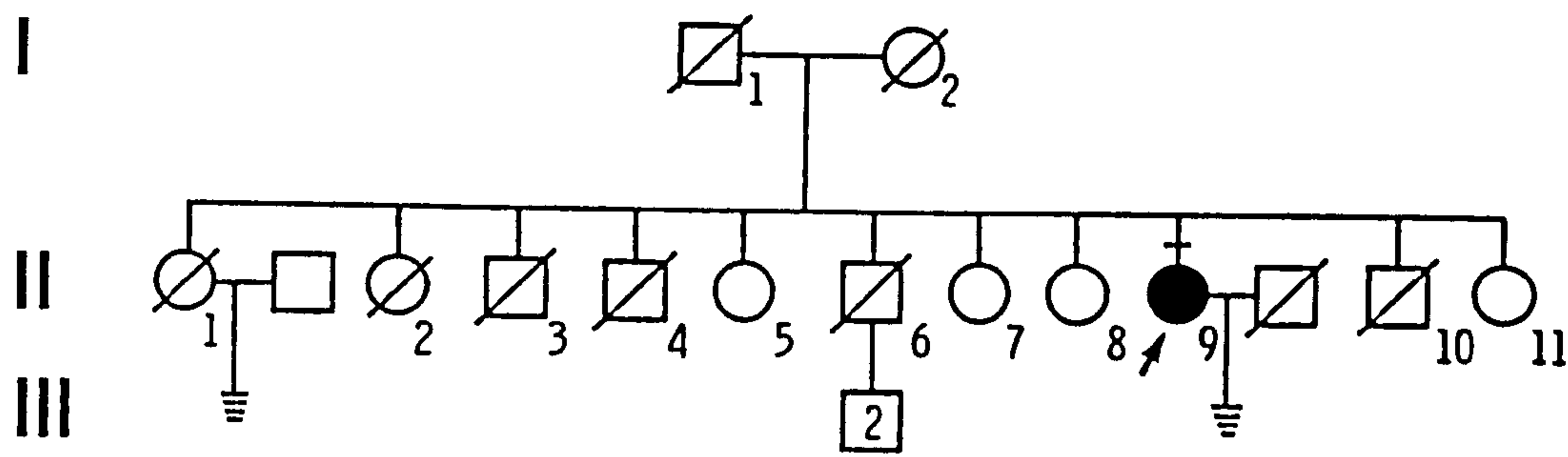
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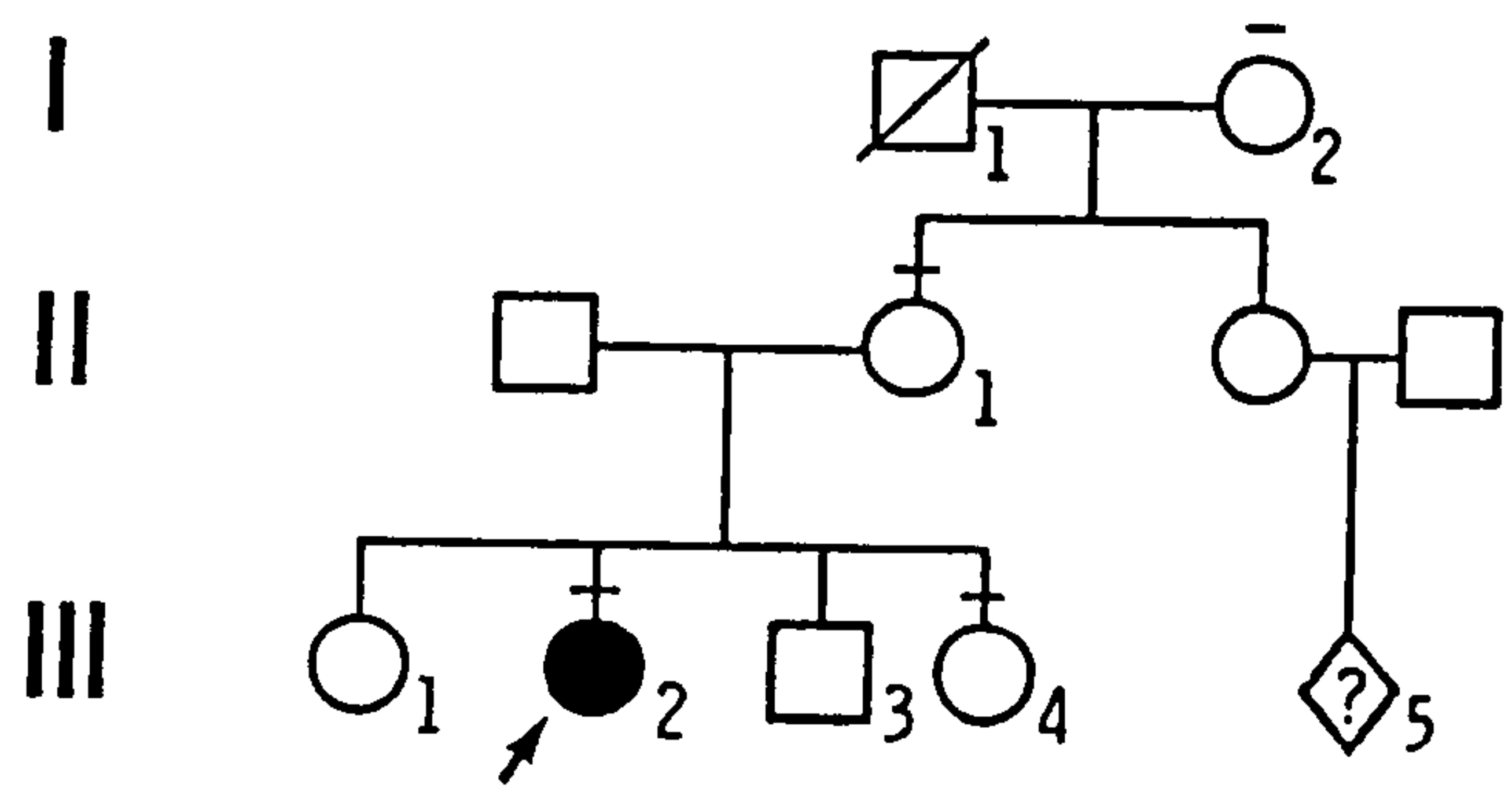
NF4



NF5

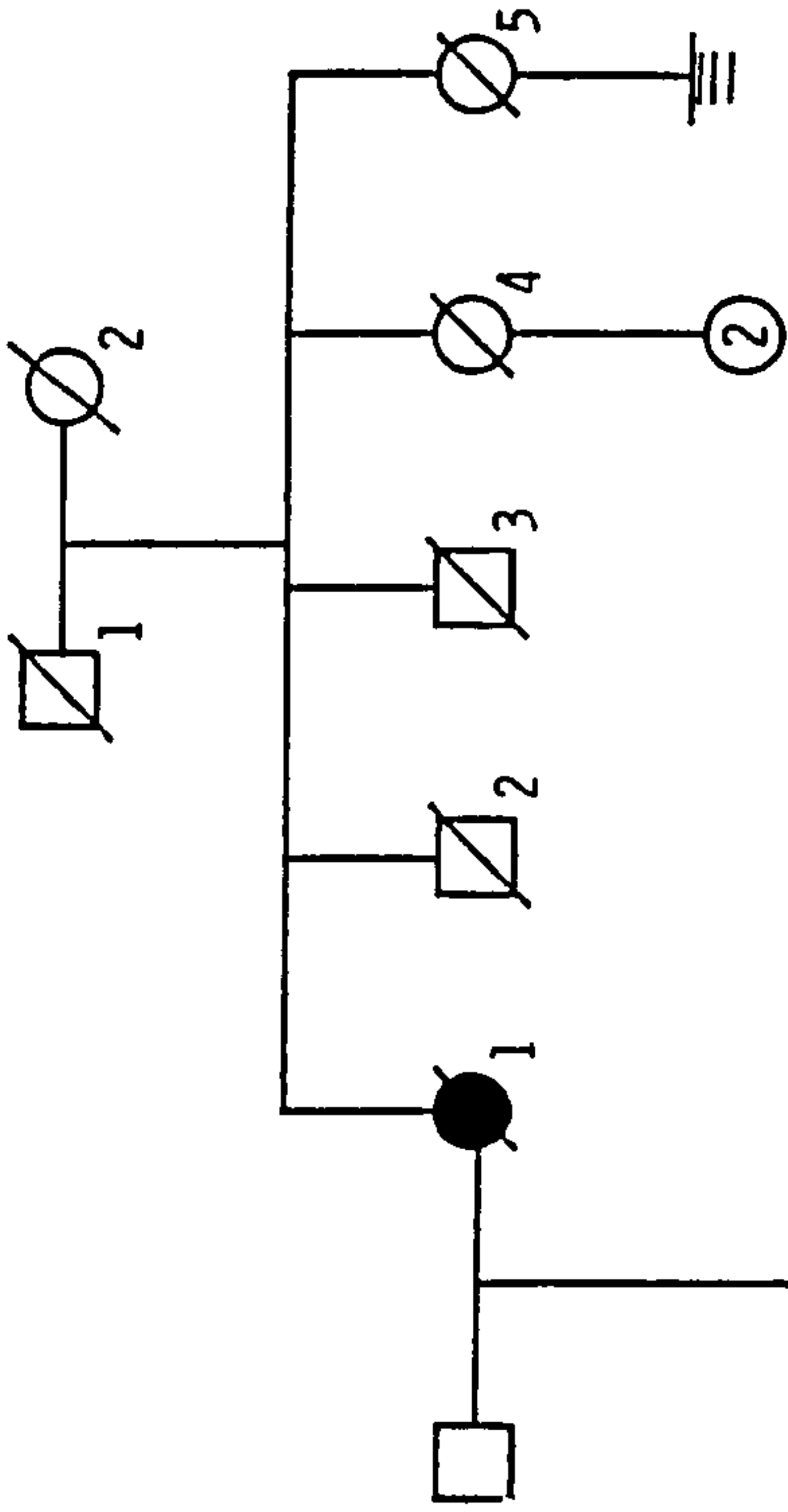


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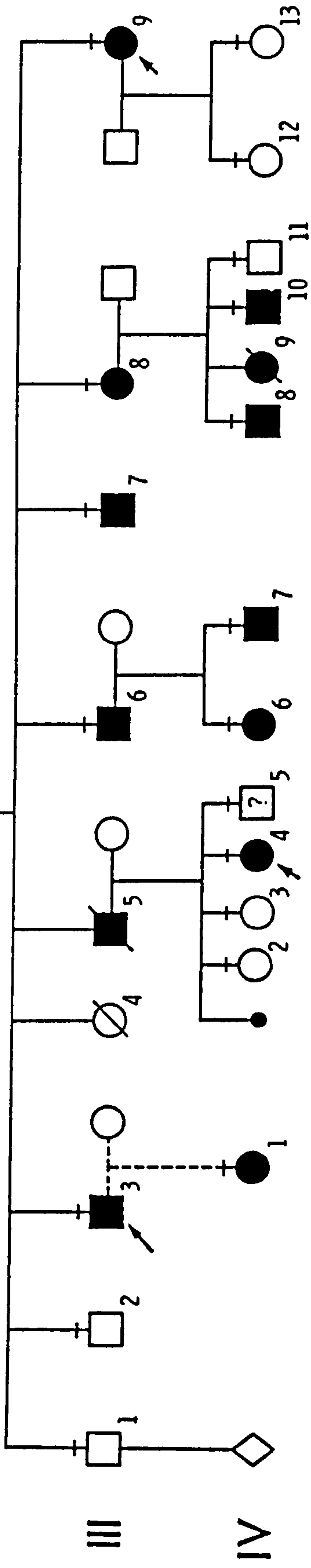


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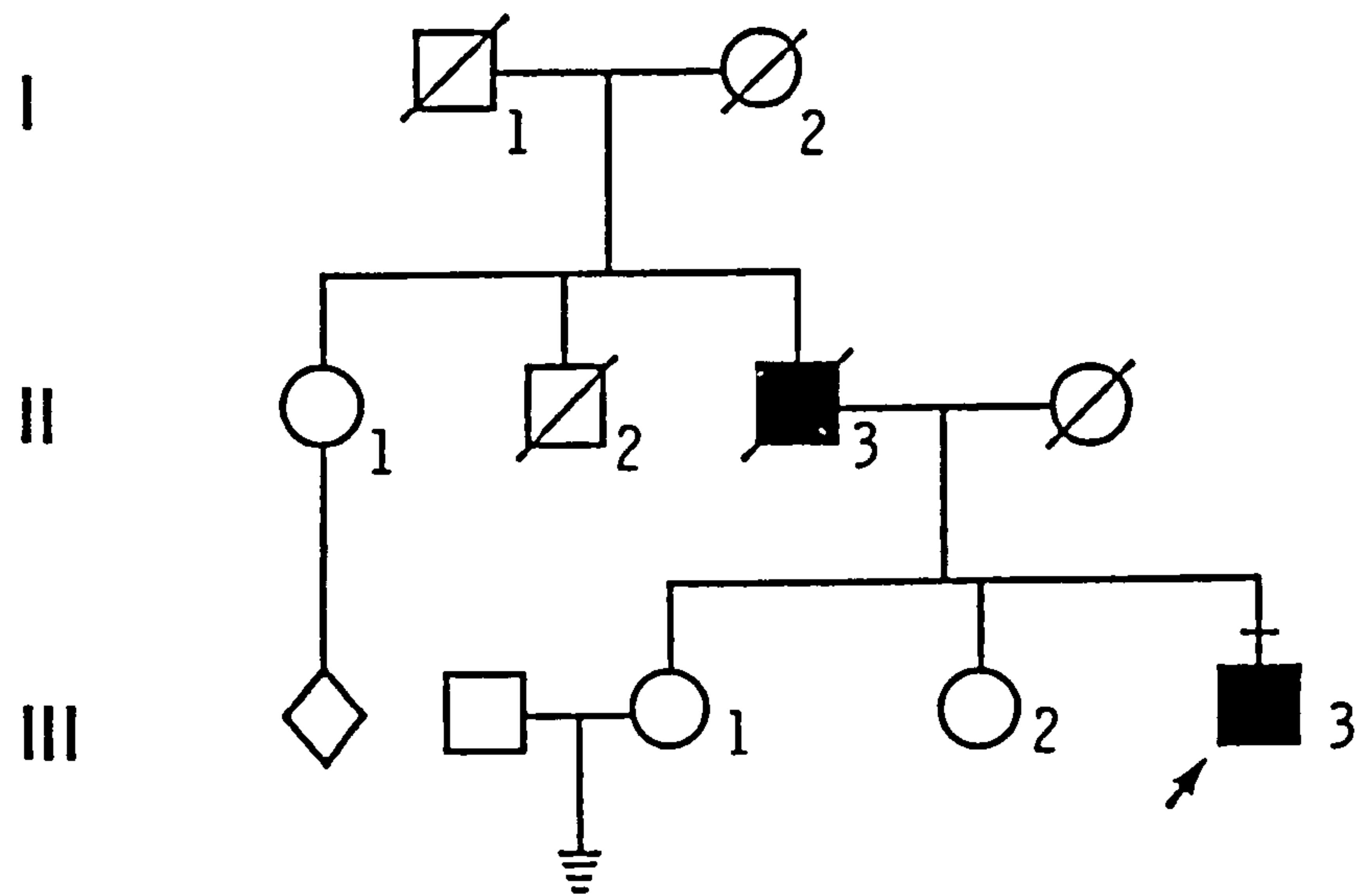
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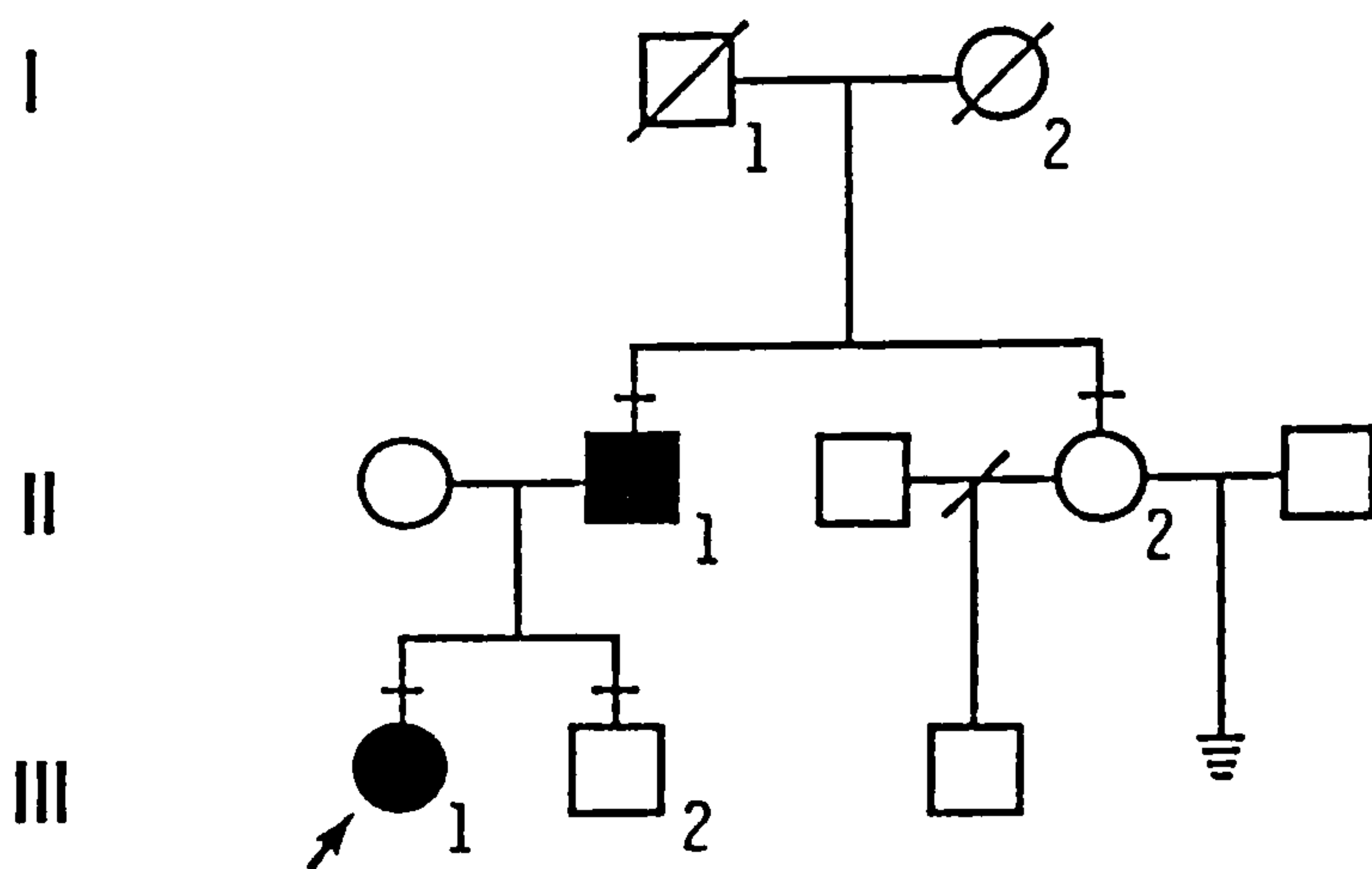
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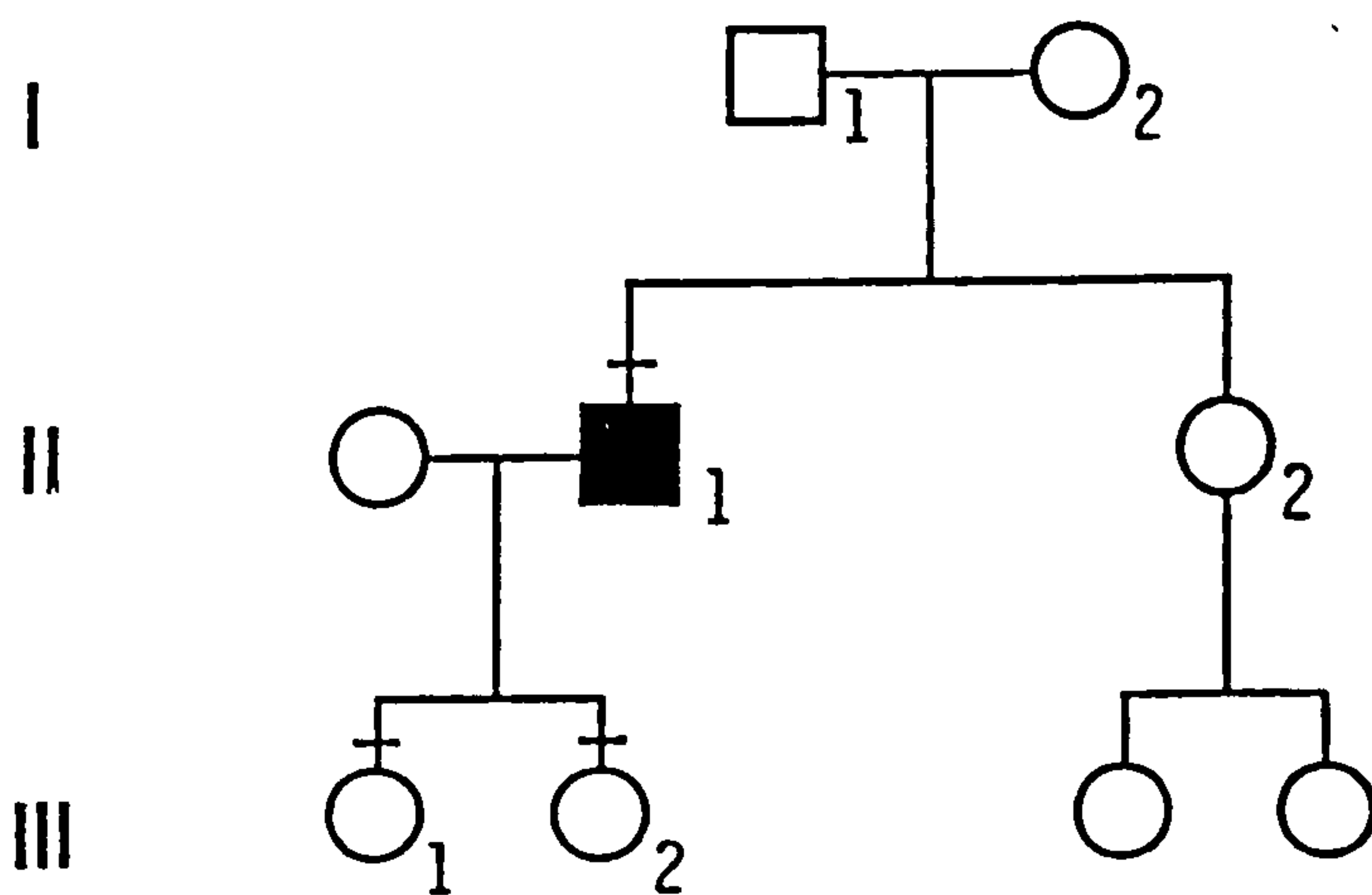
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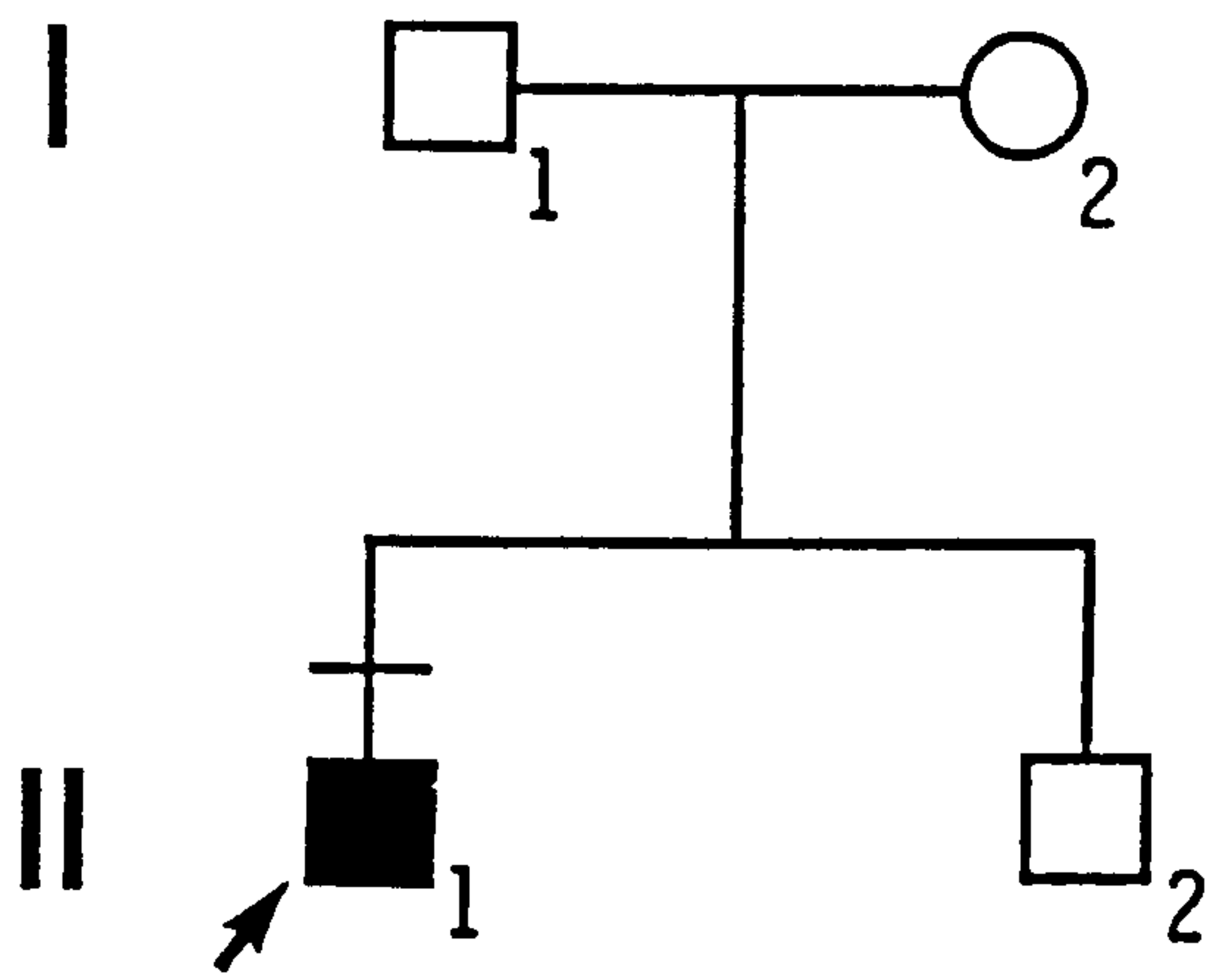
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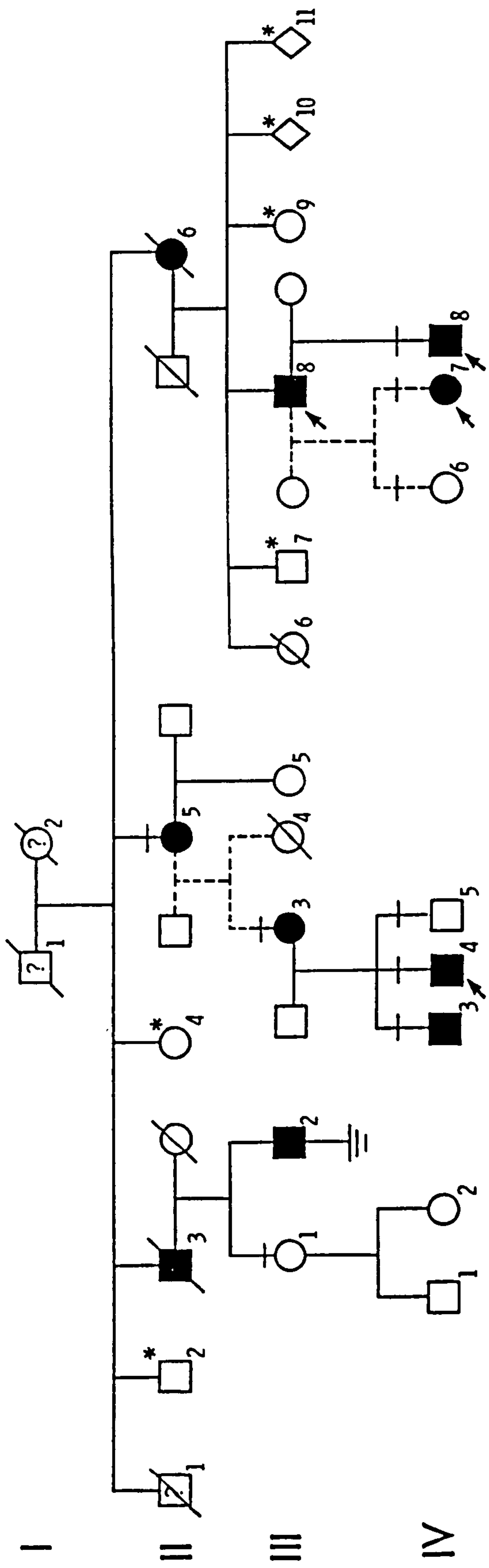
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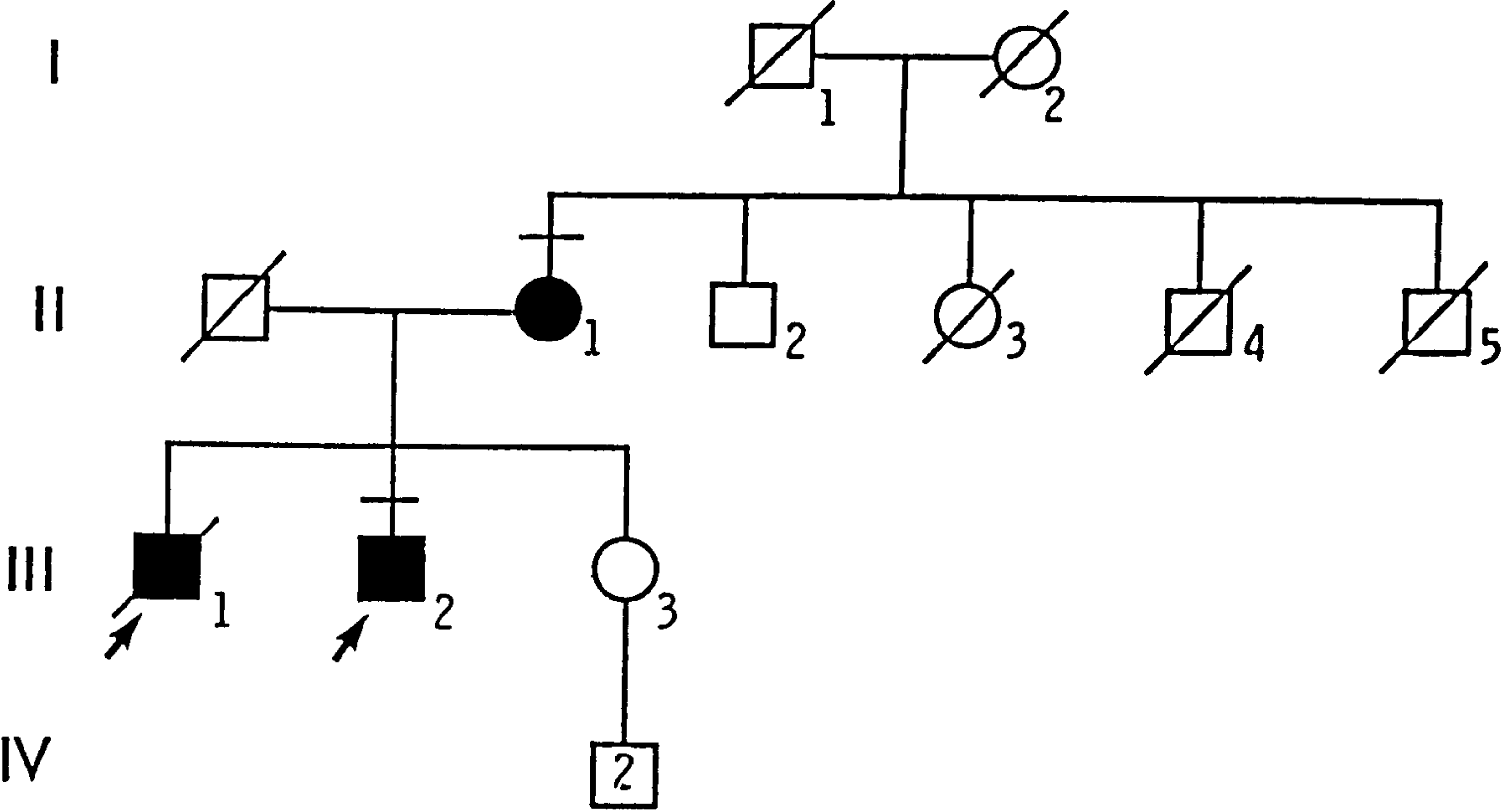
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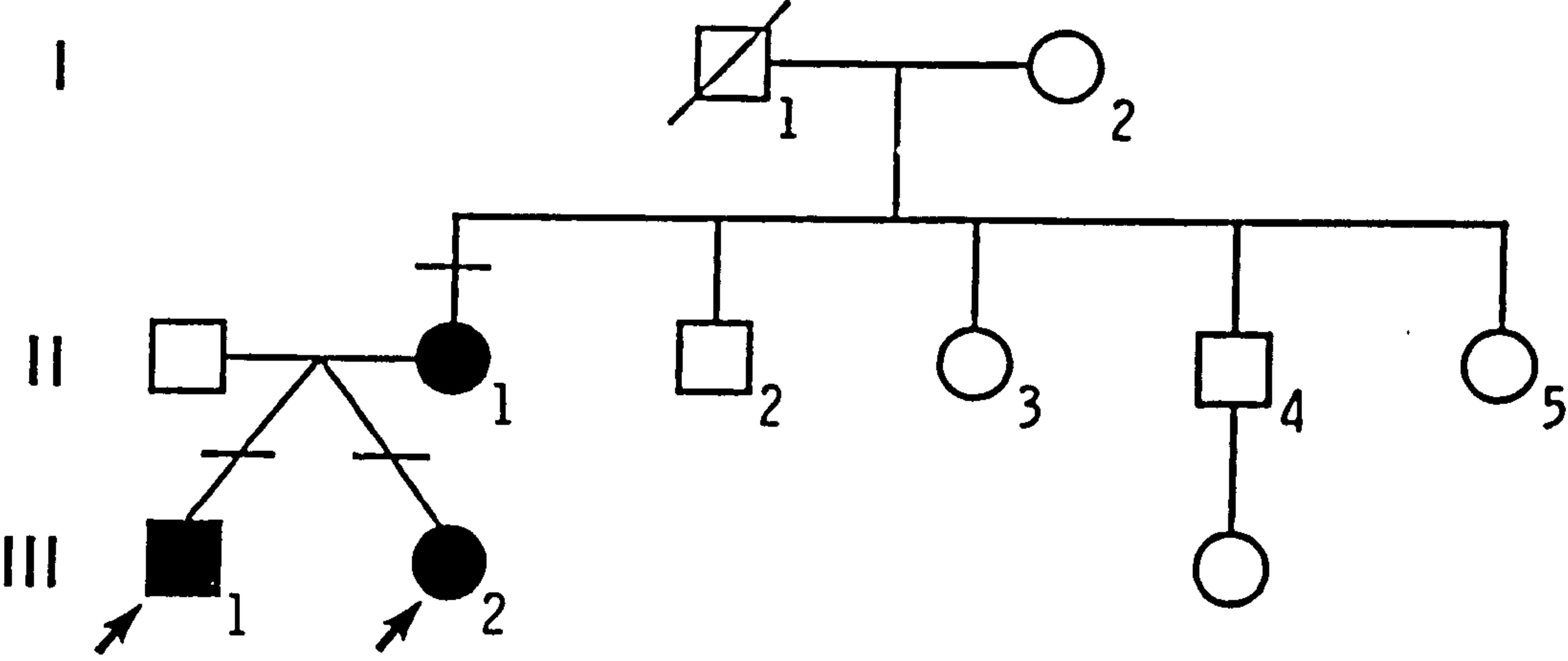
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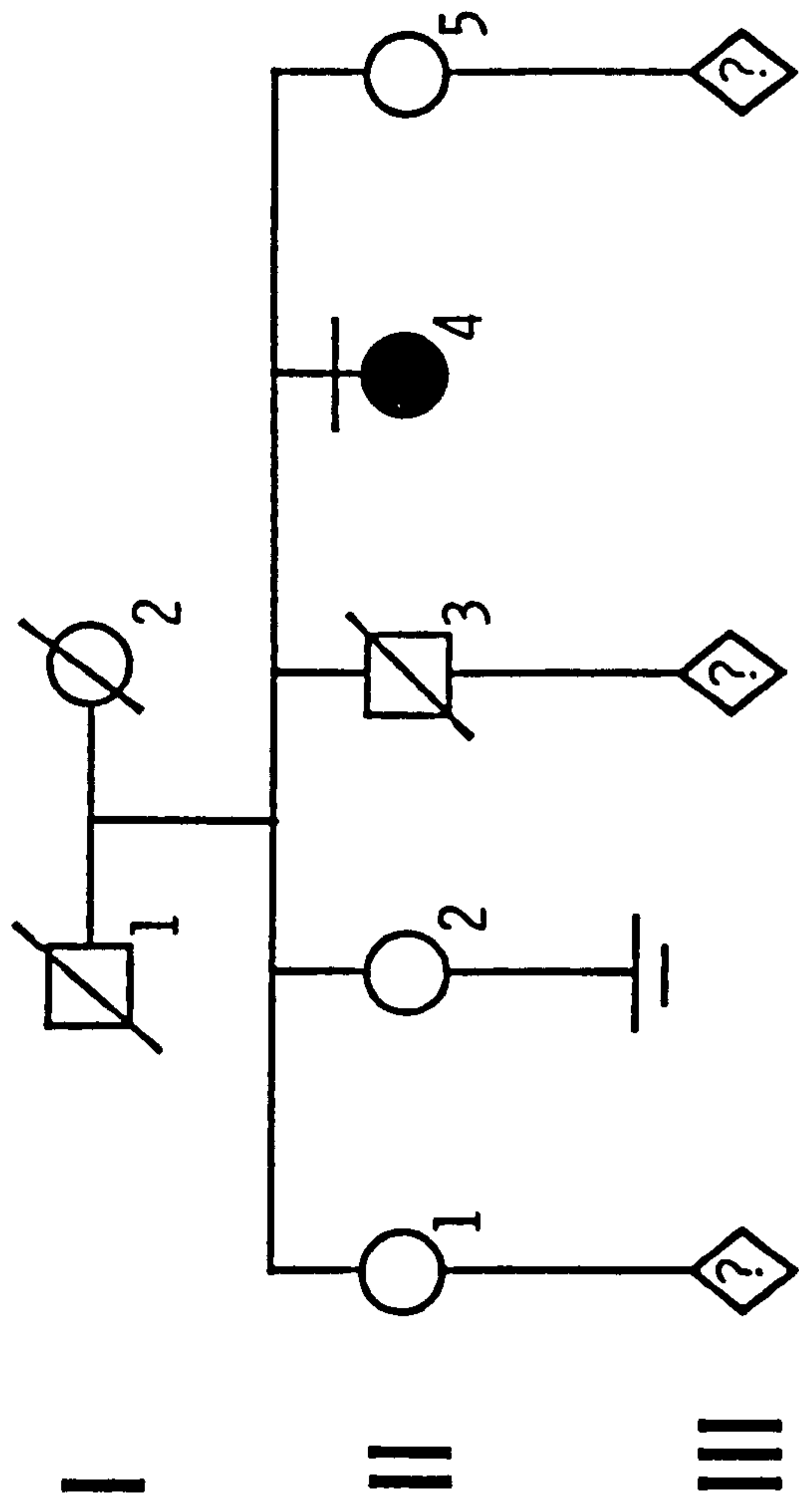
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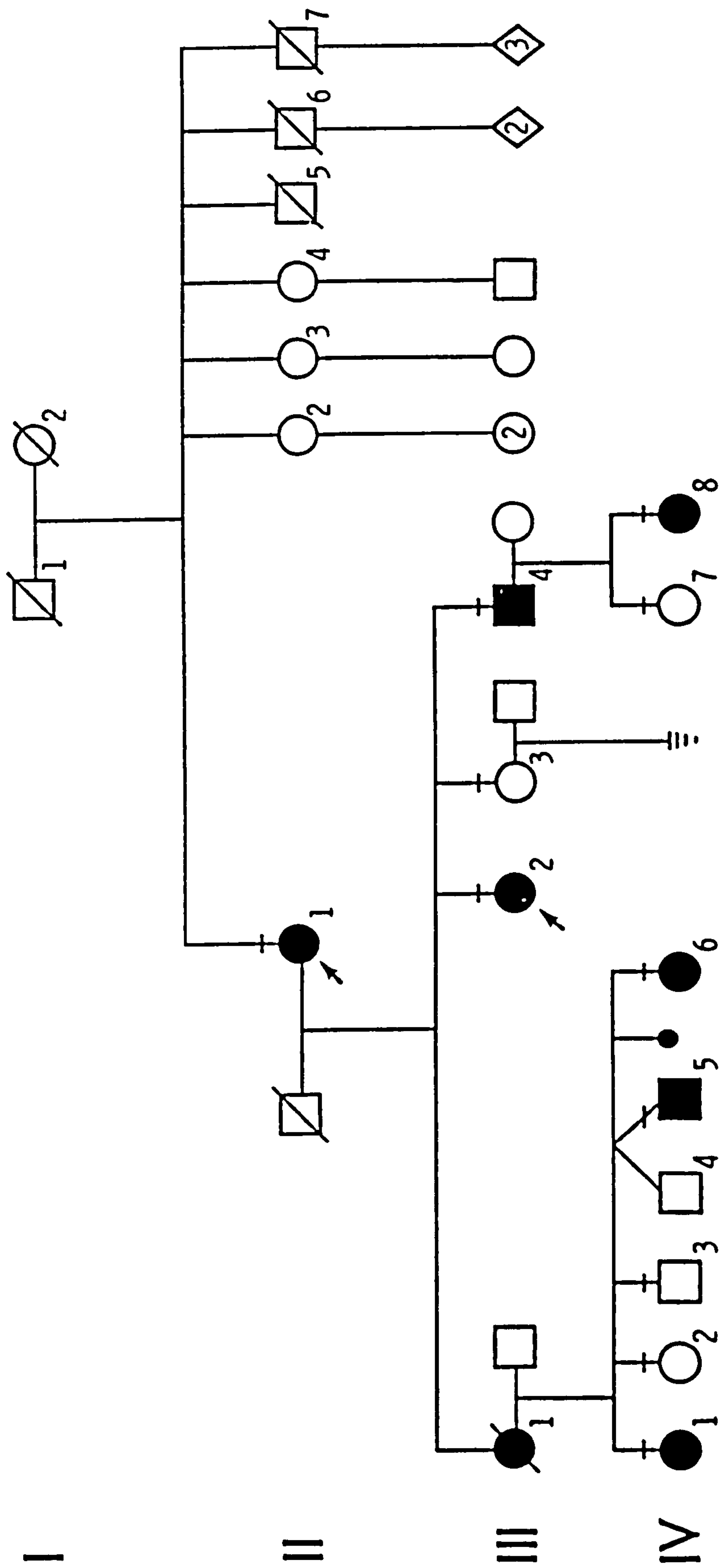
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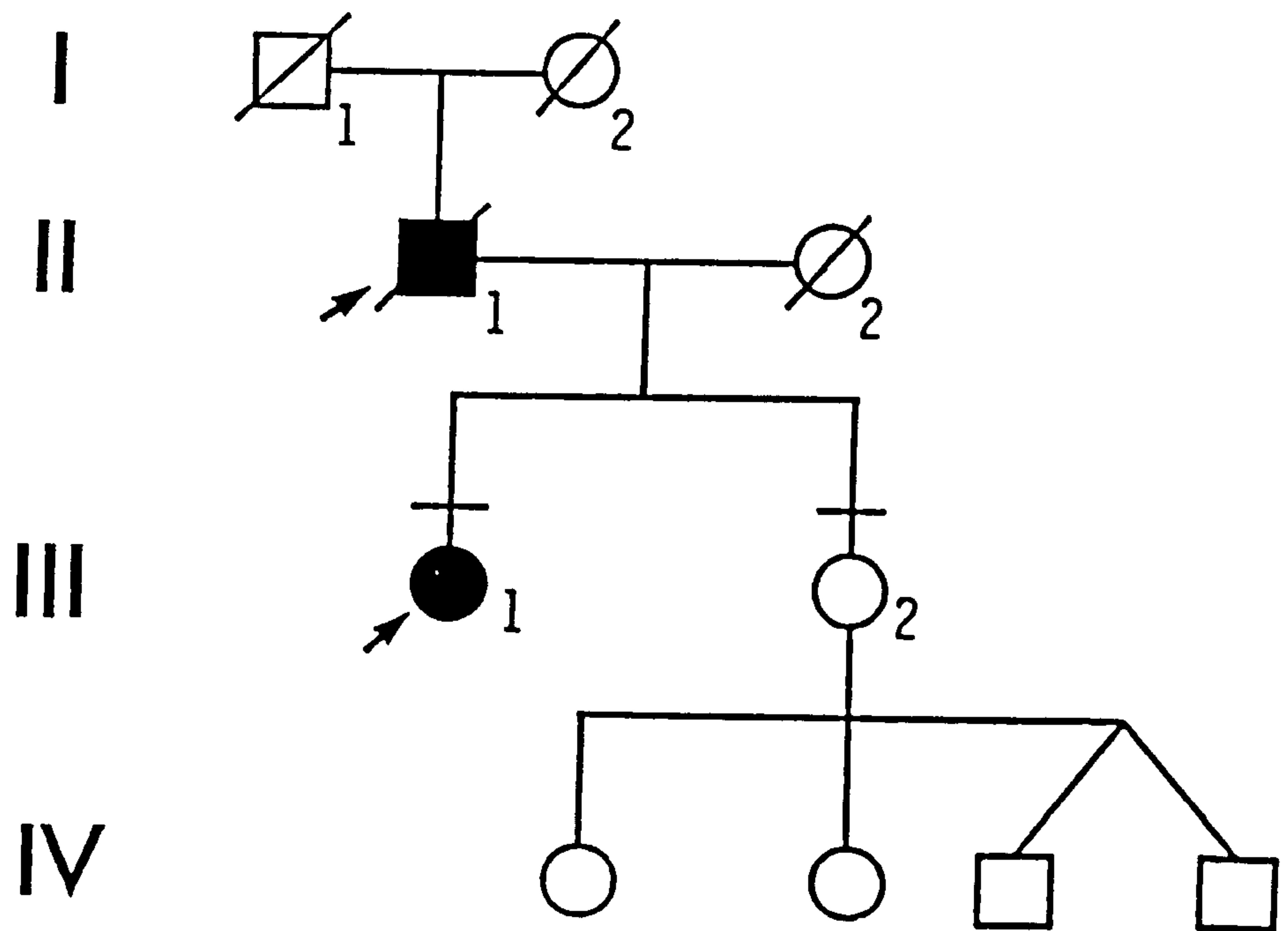
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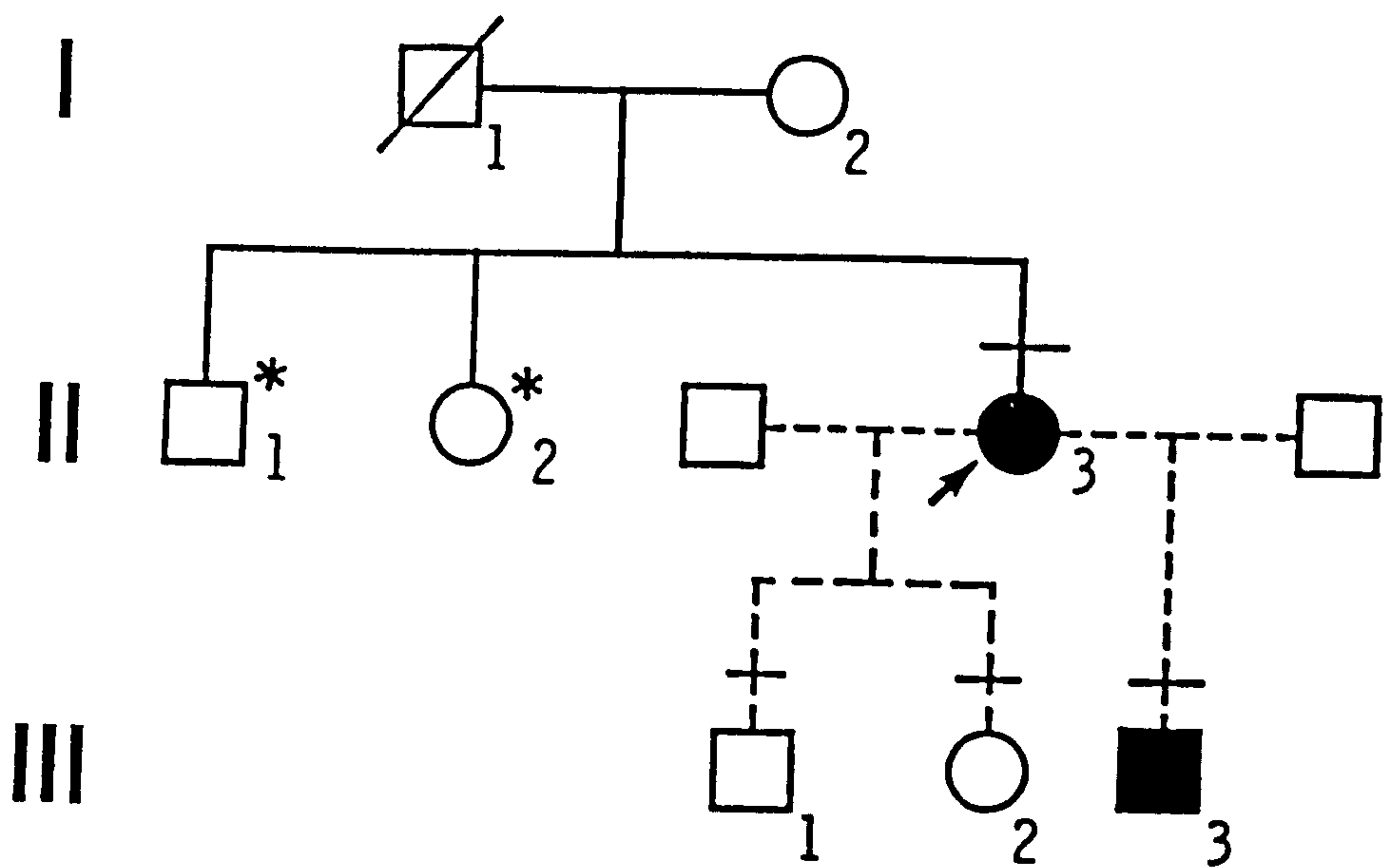
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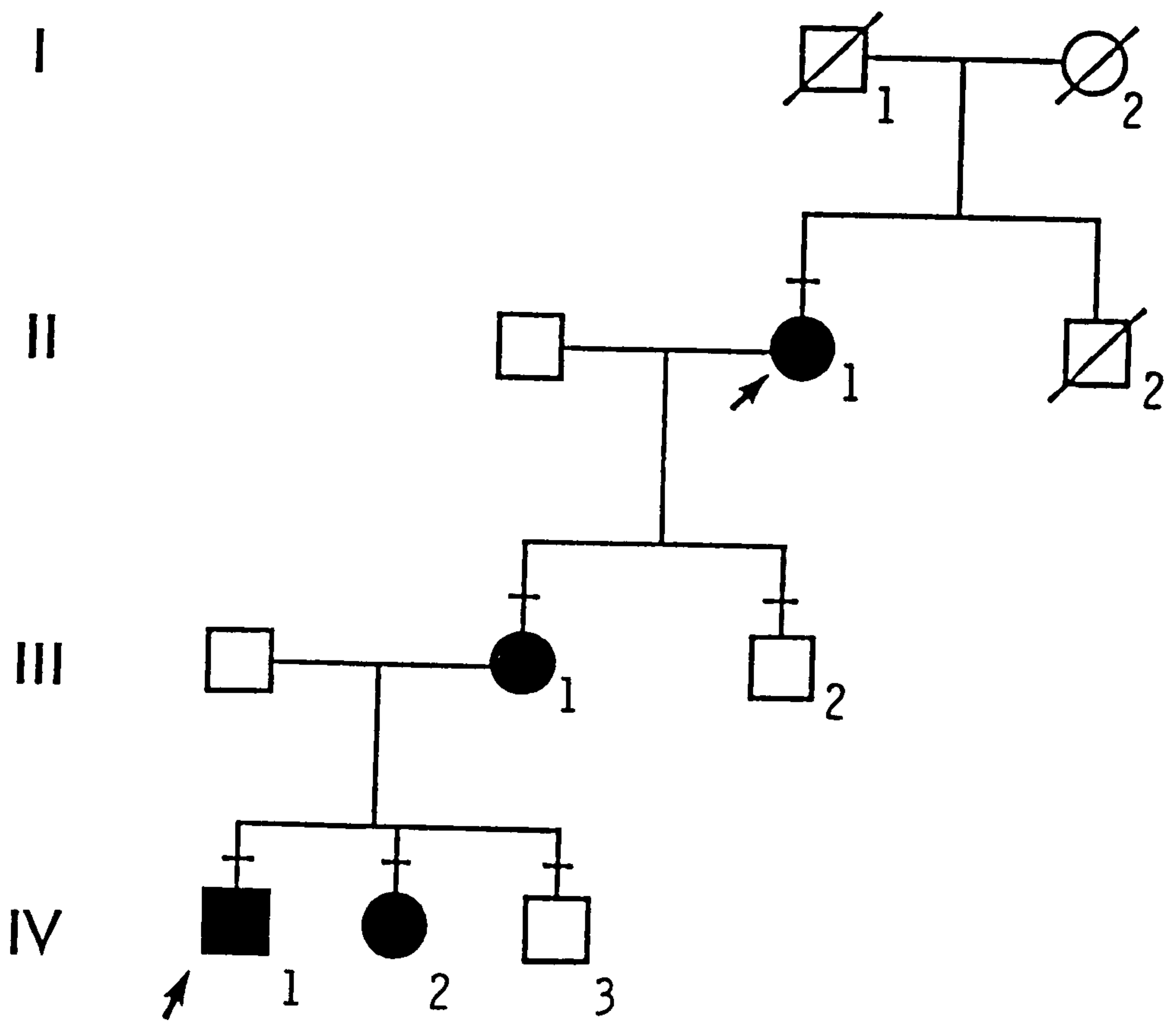
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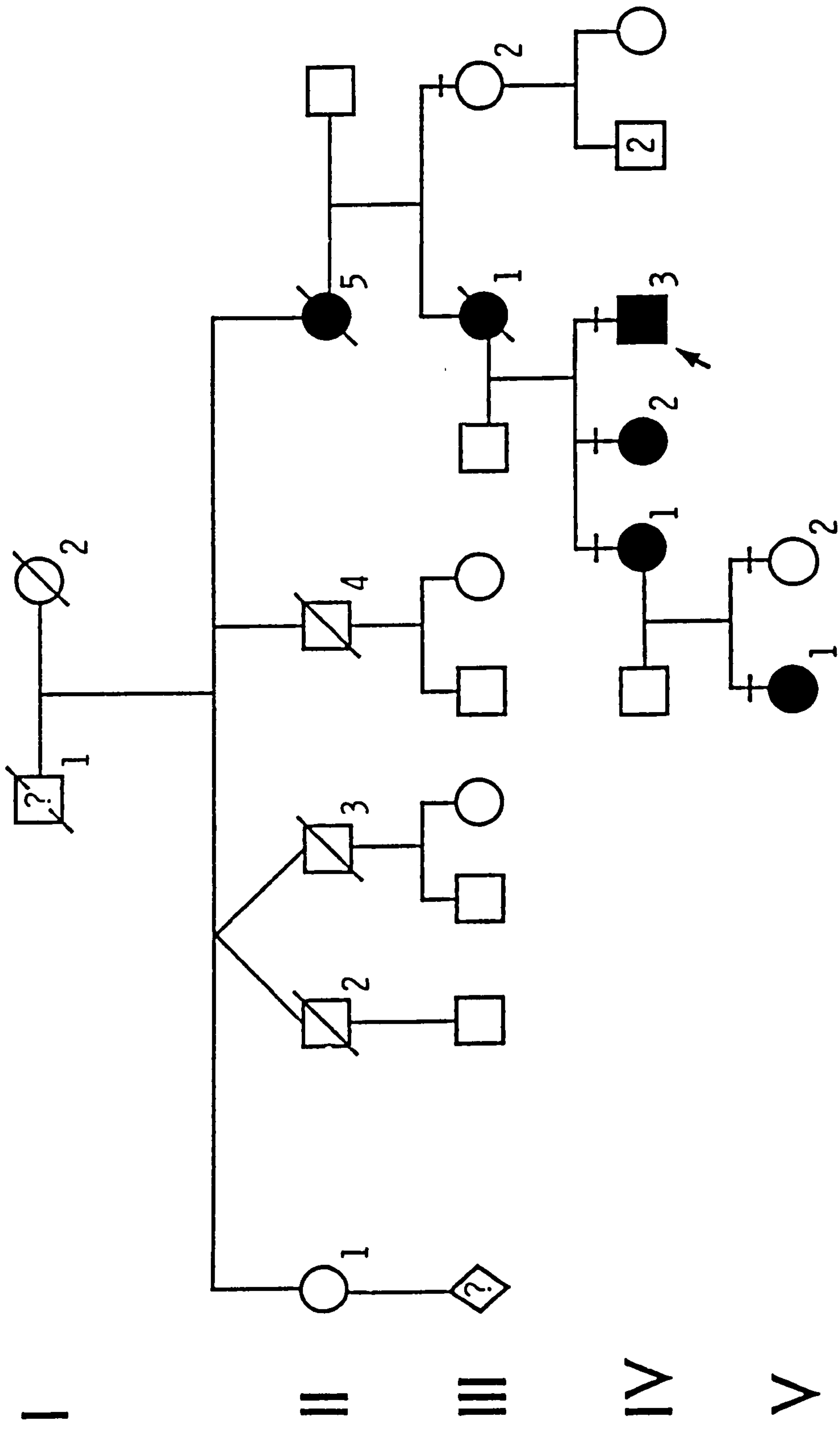
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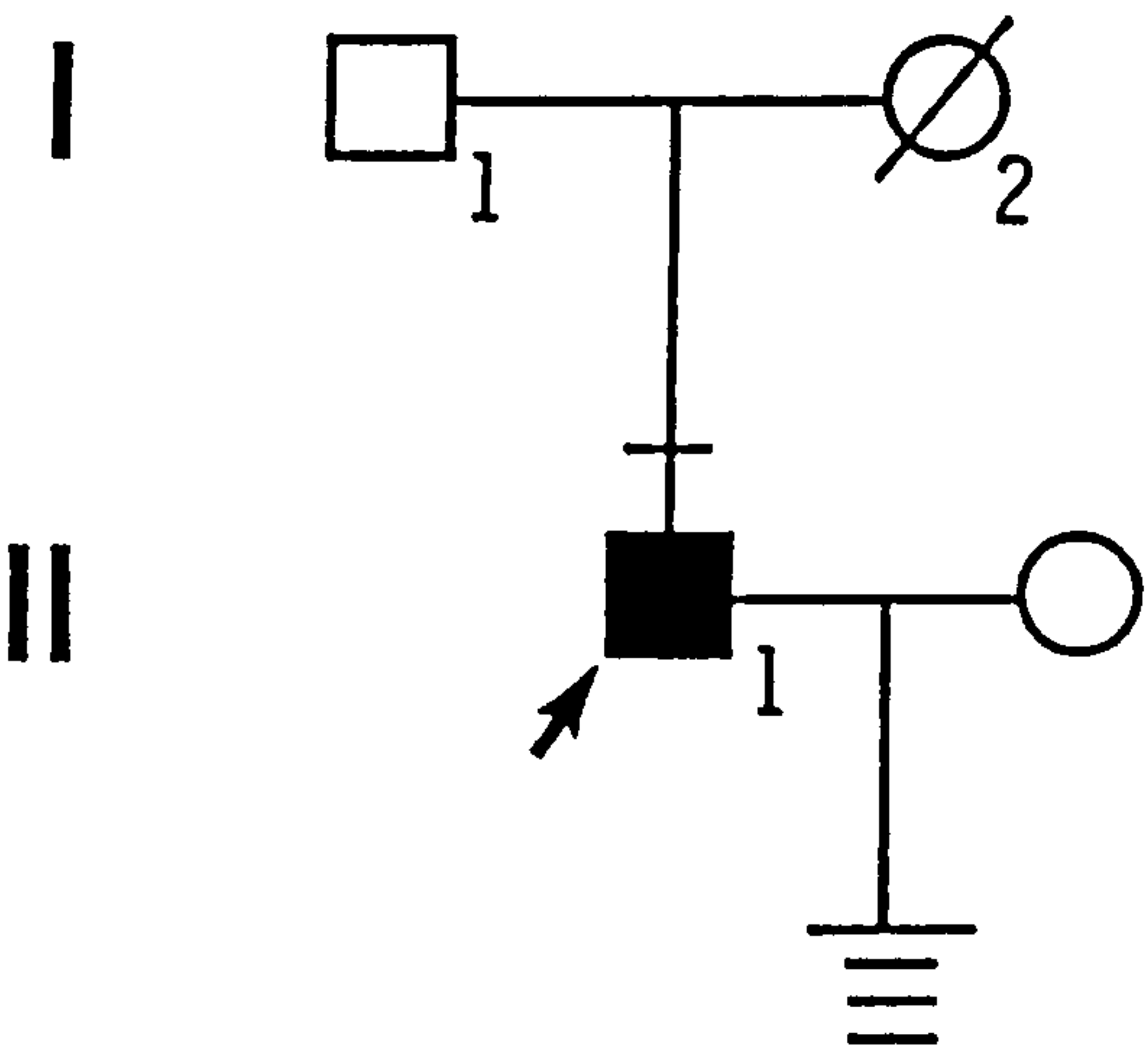
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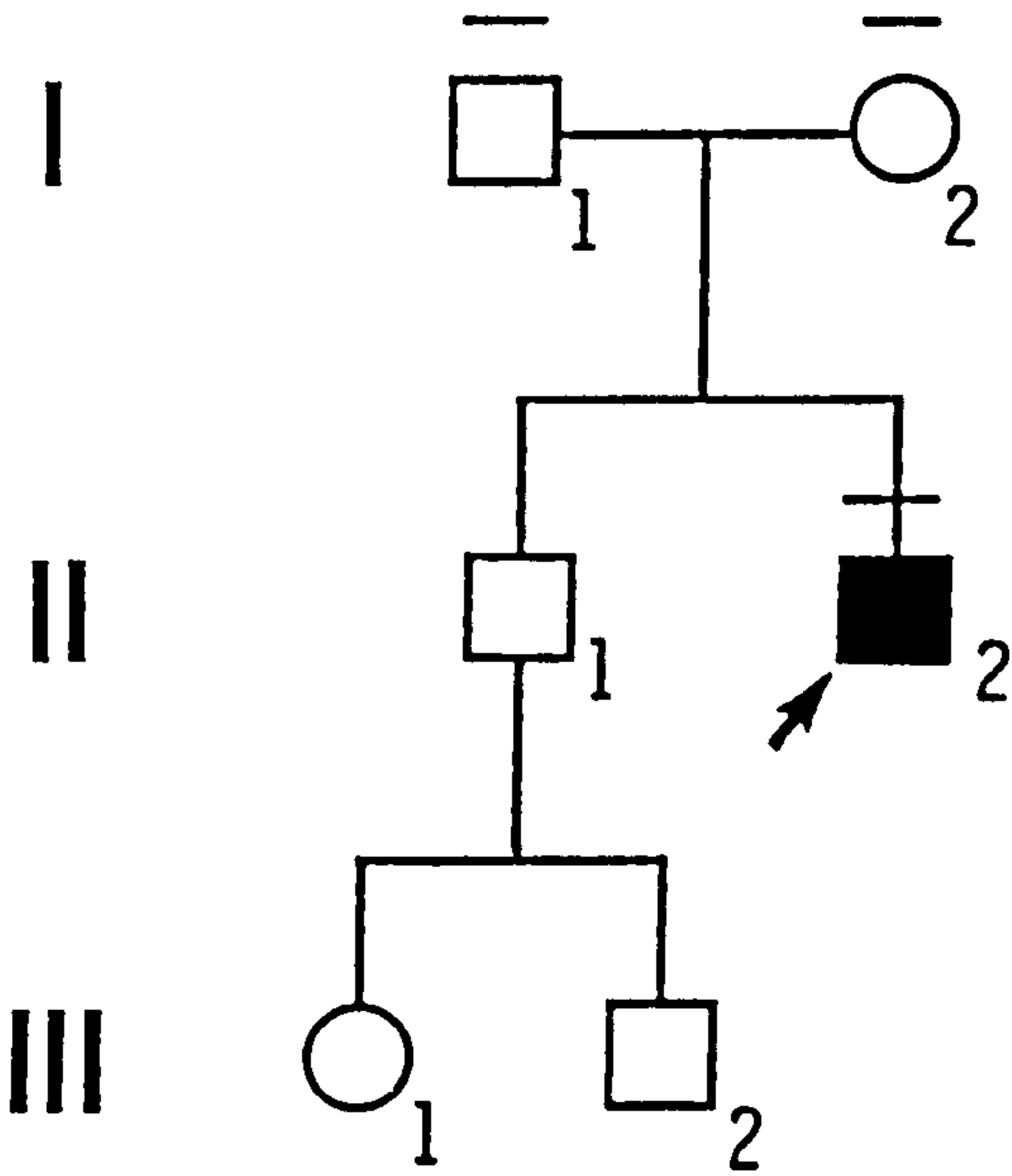
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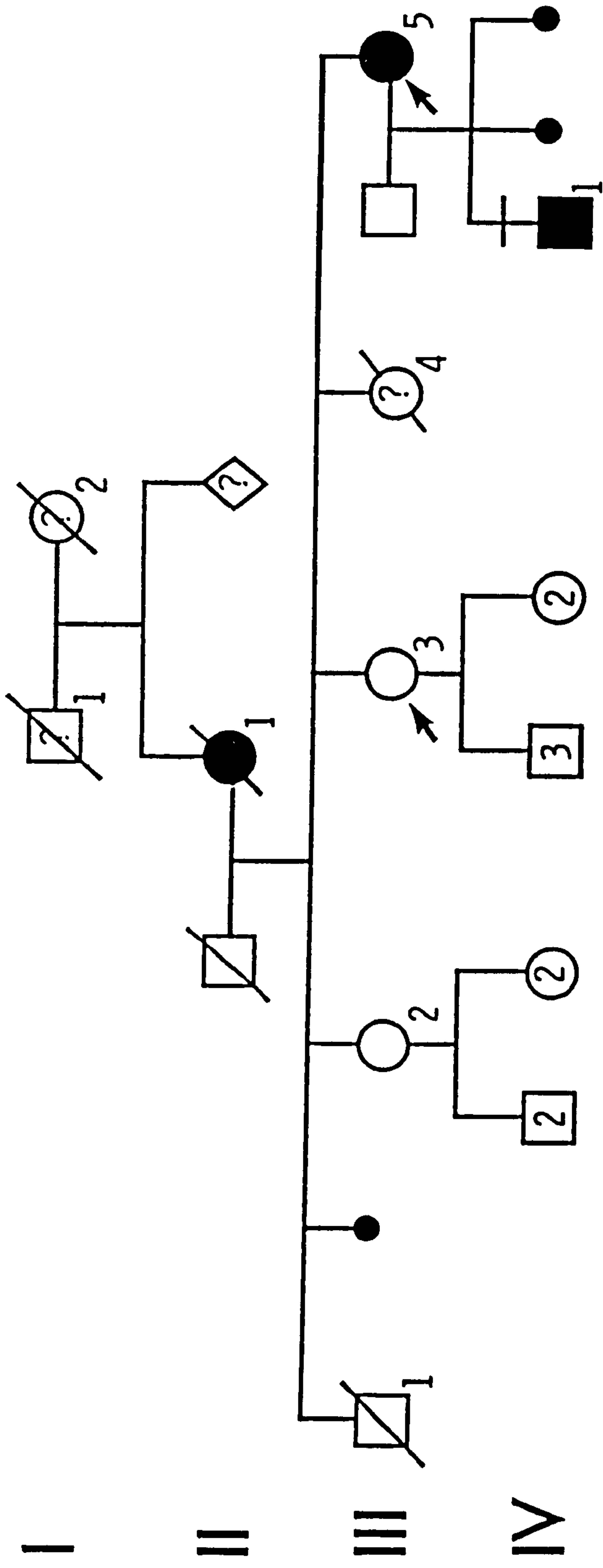
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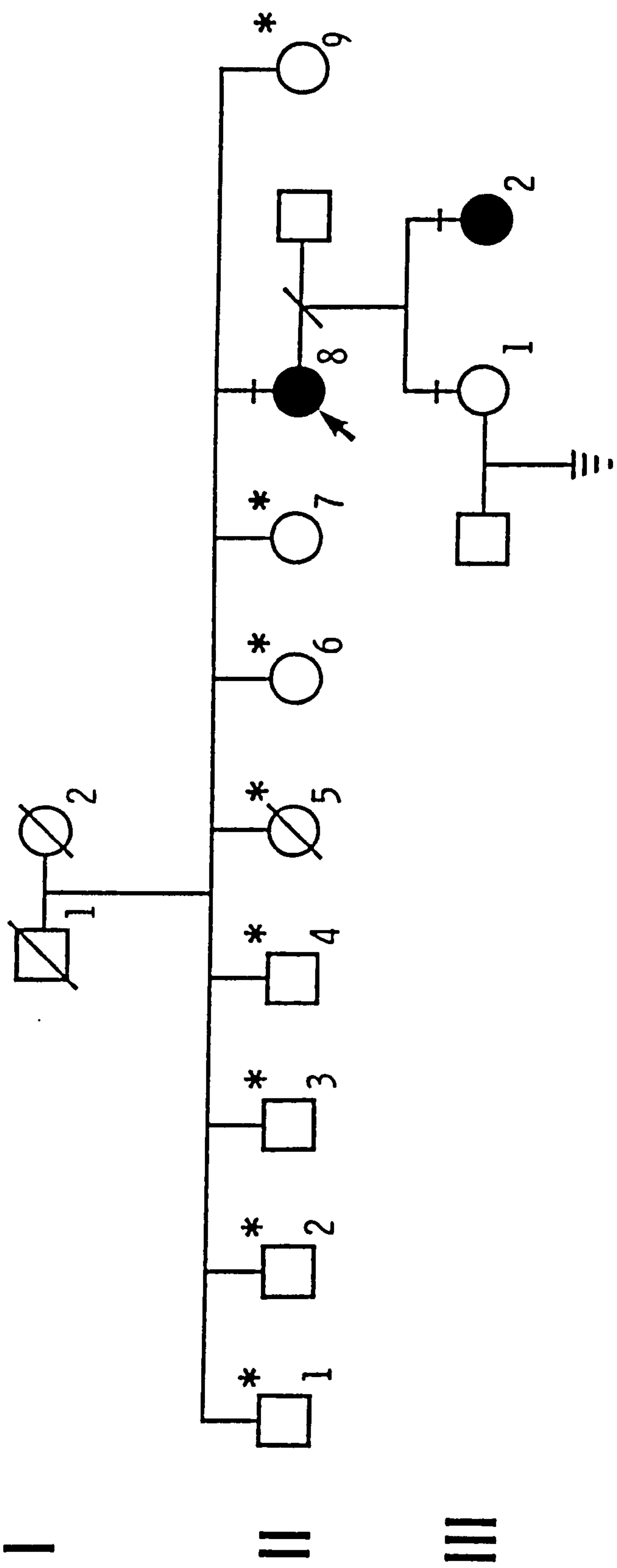
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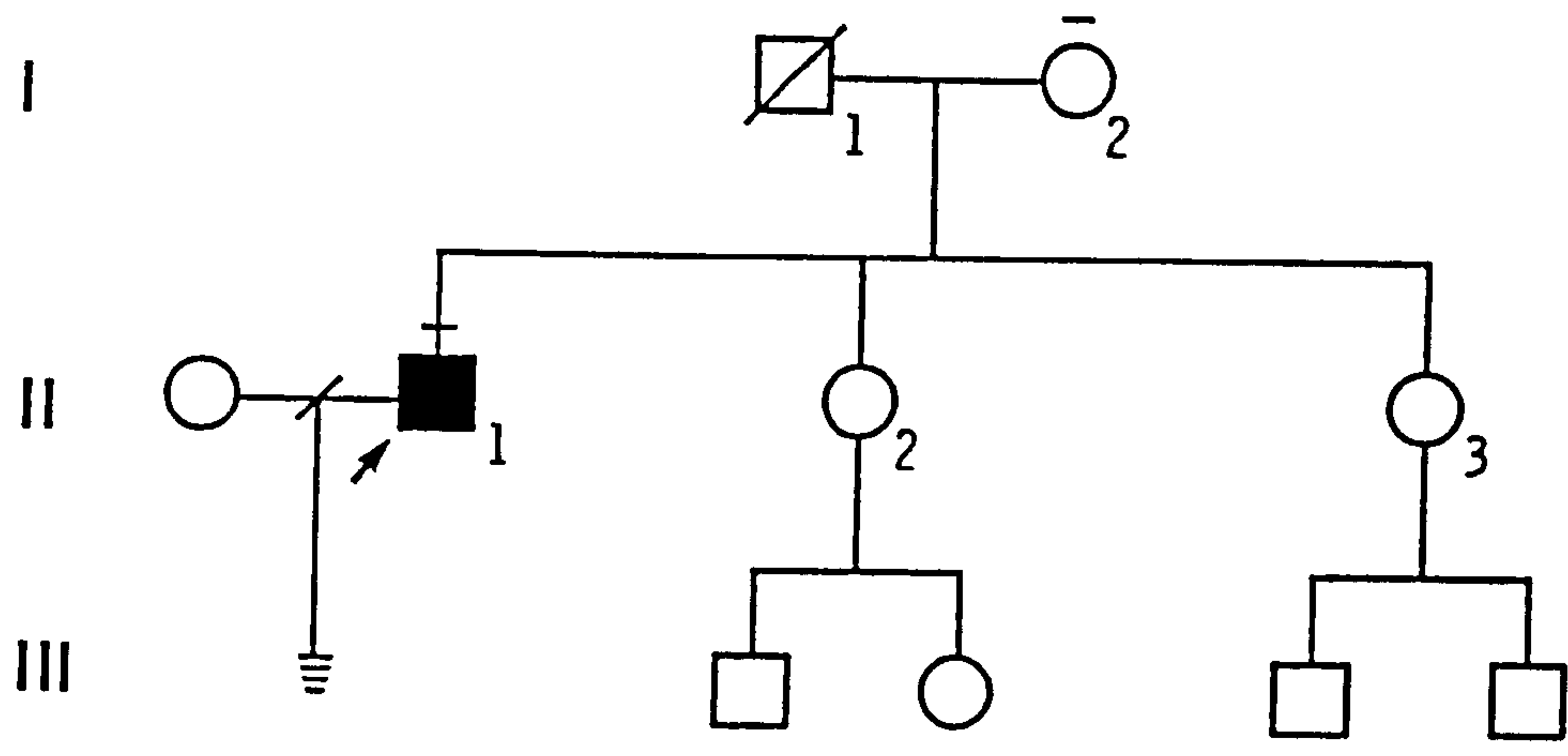
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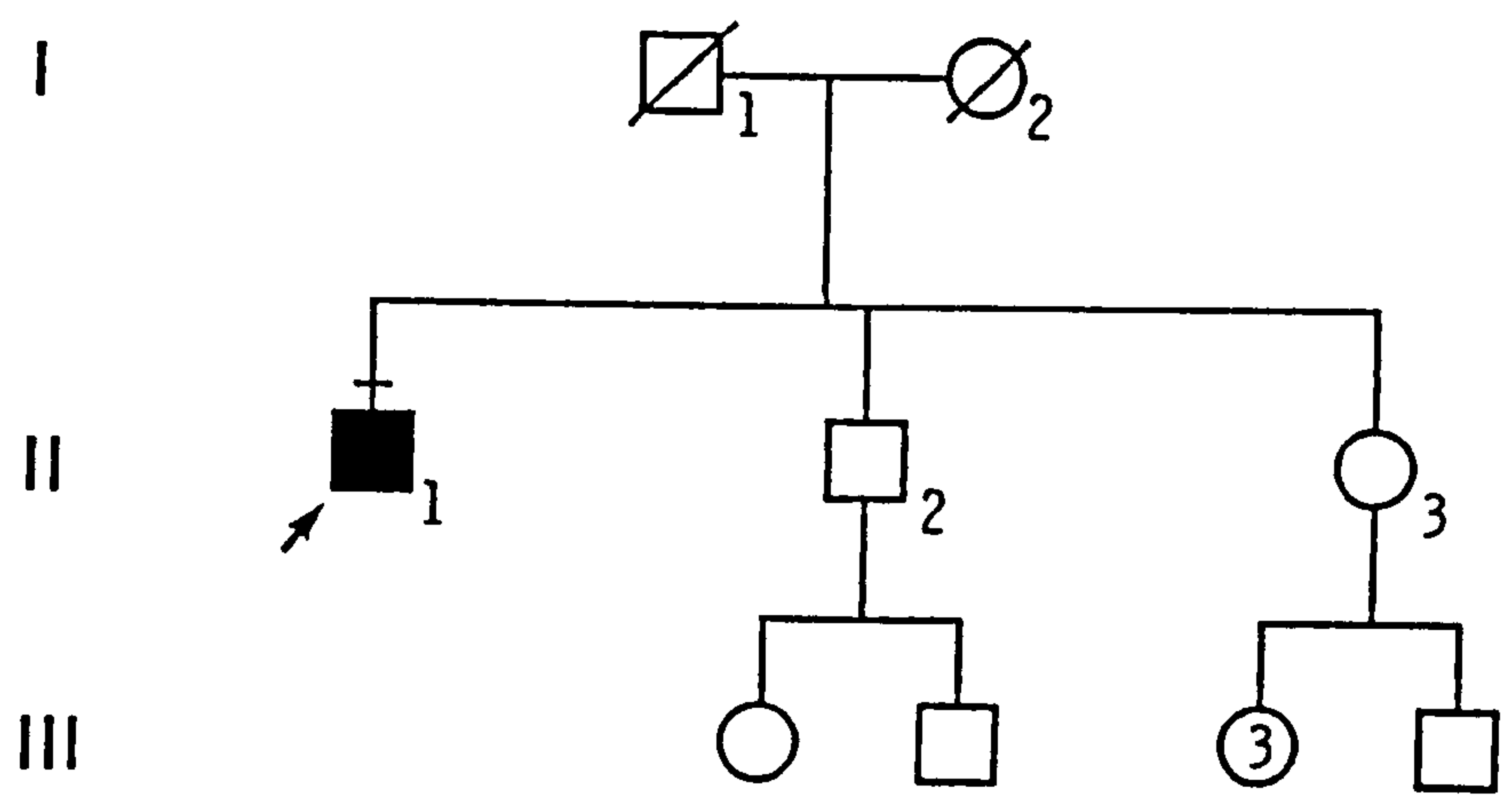
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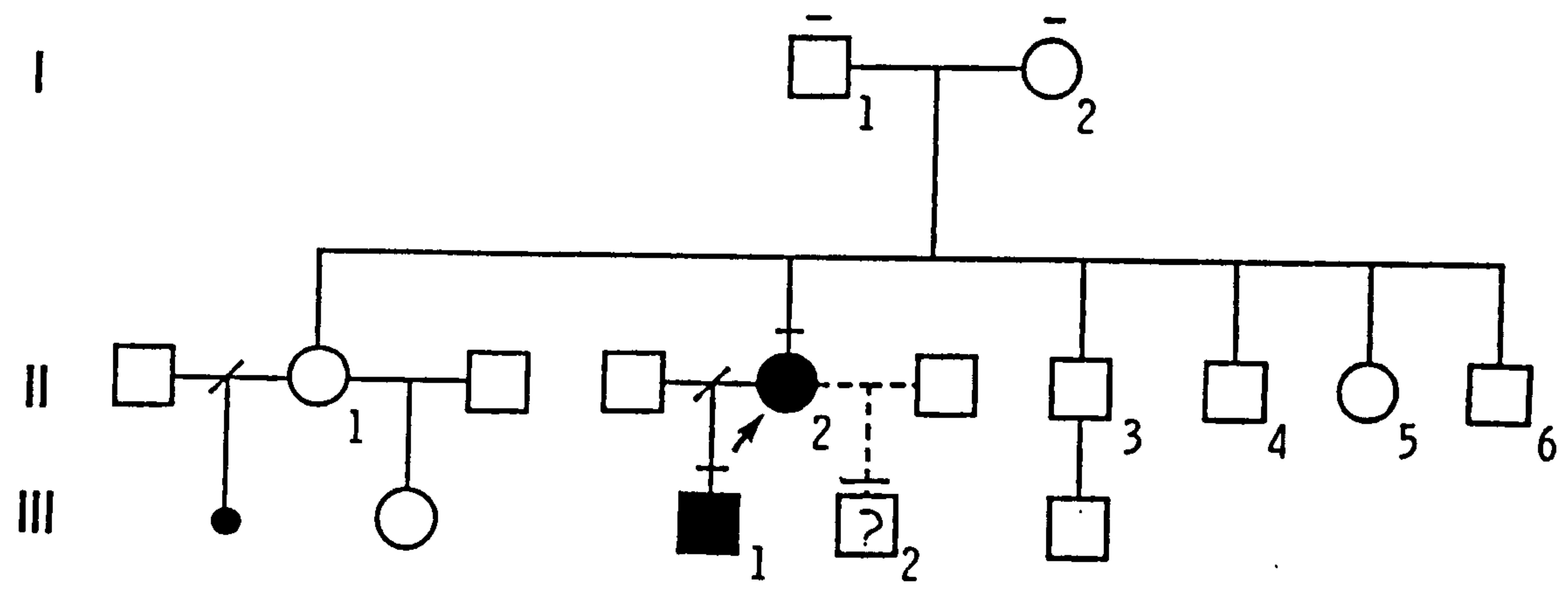
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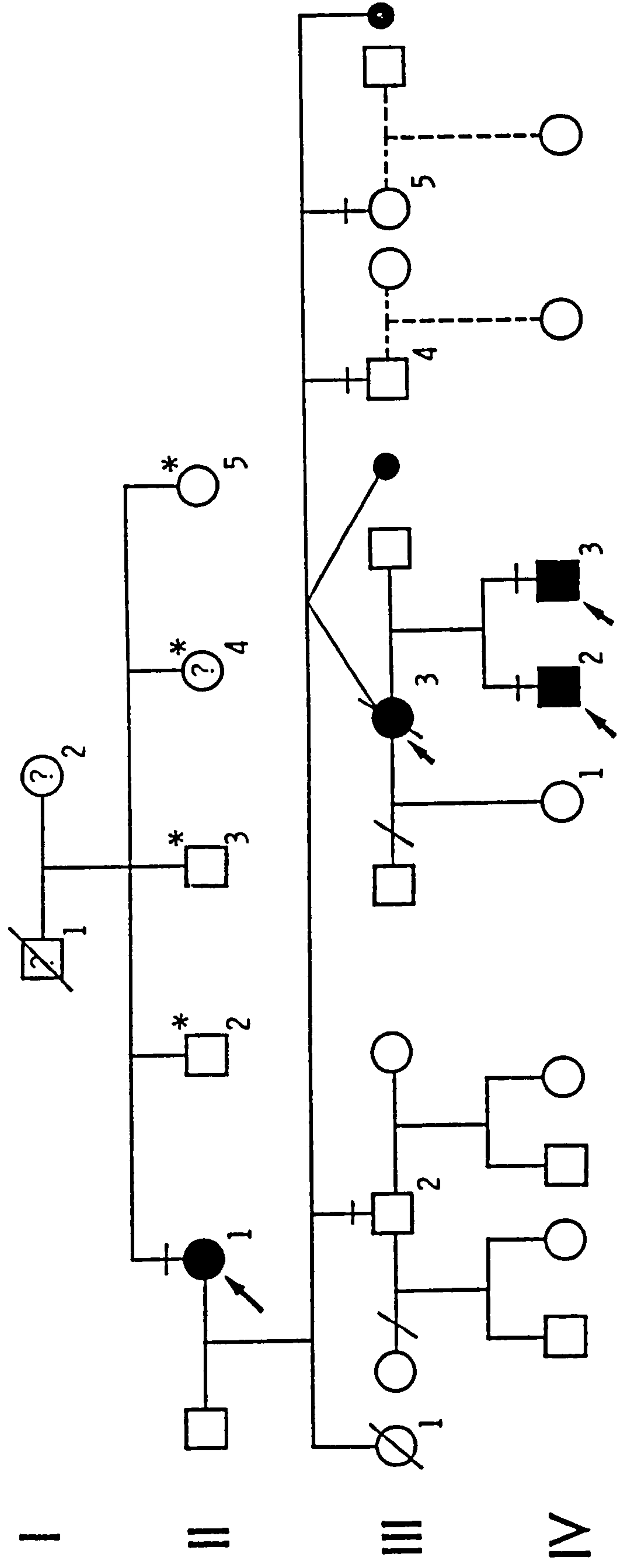
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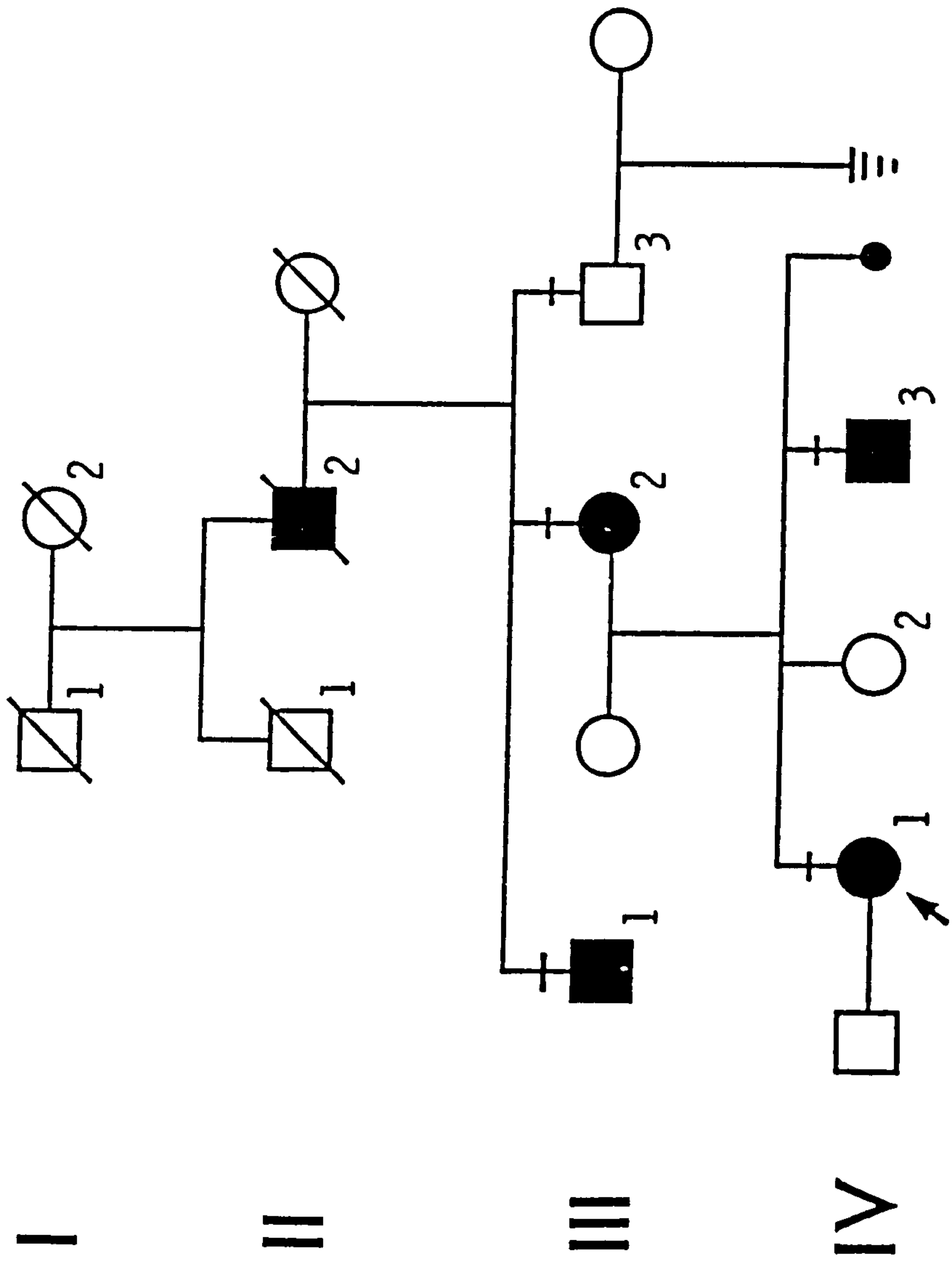
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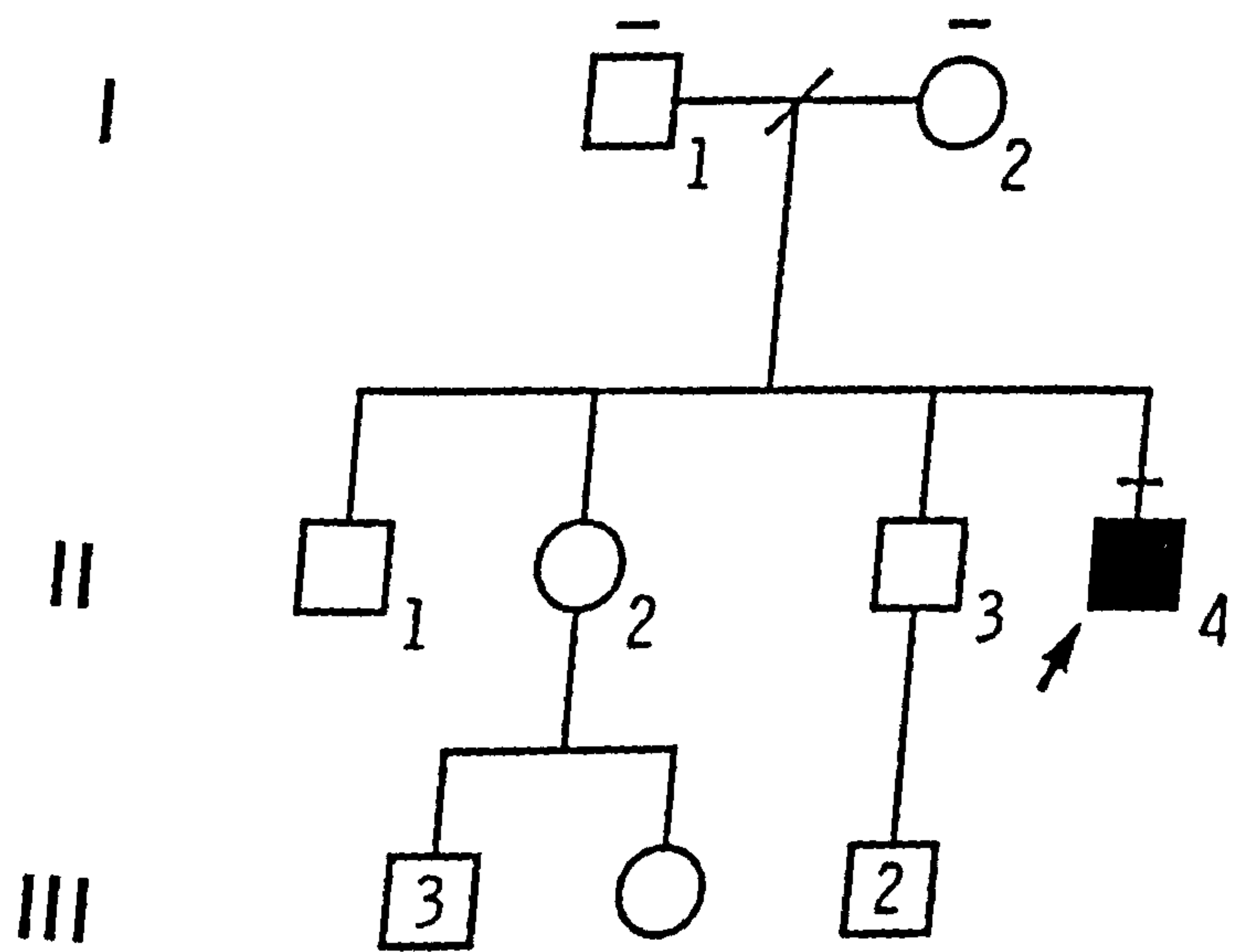
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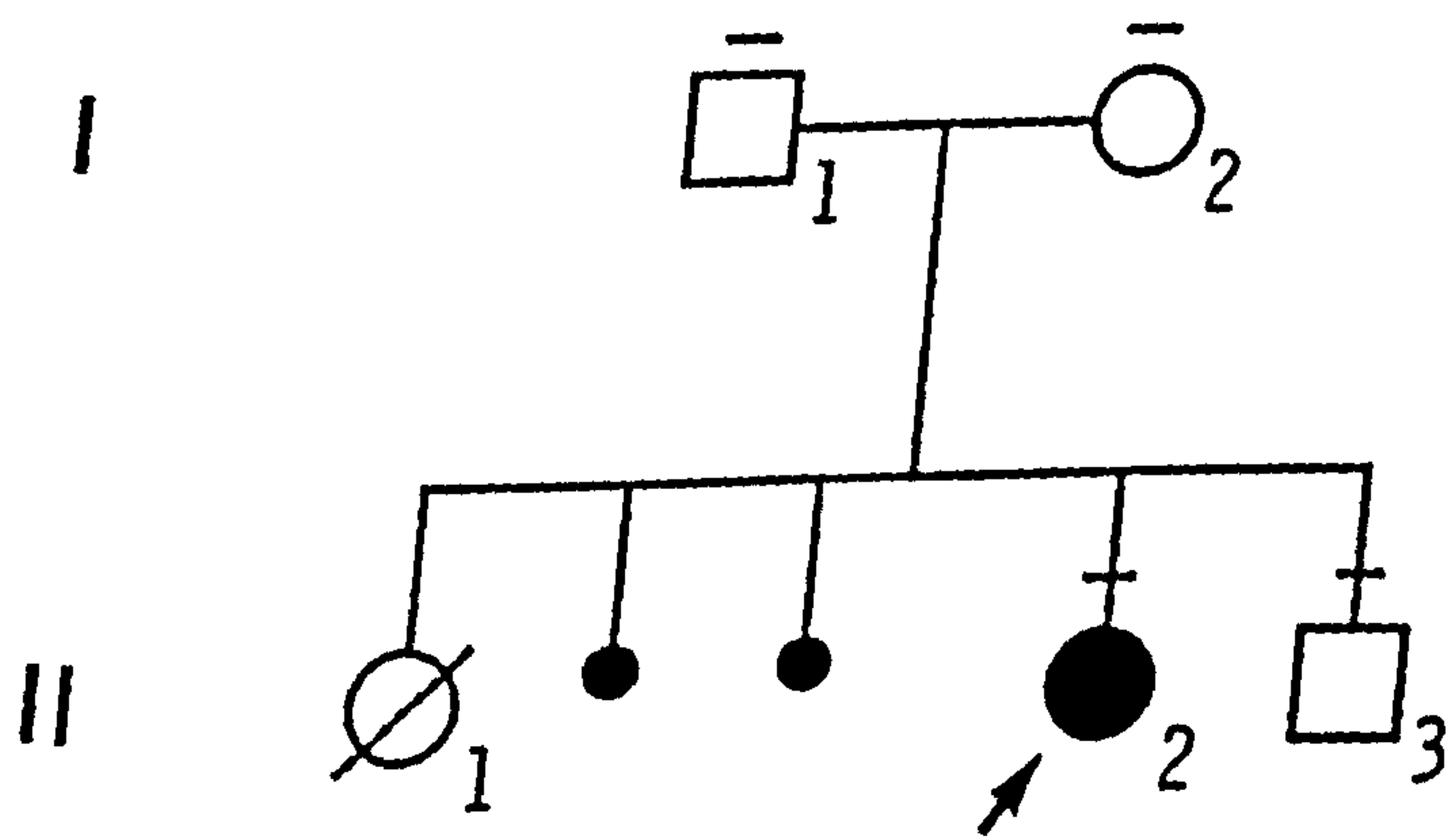
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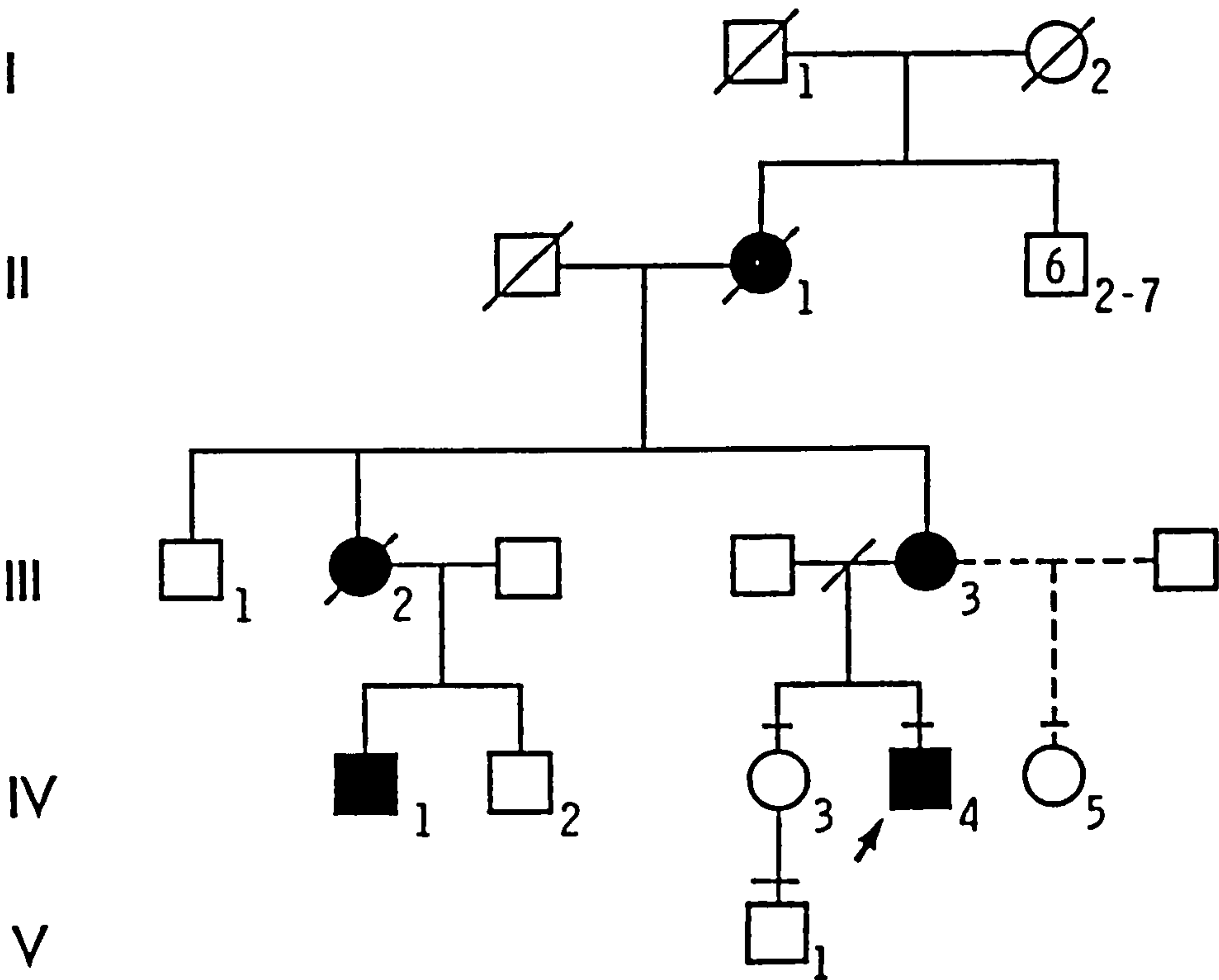
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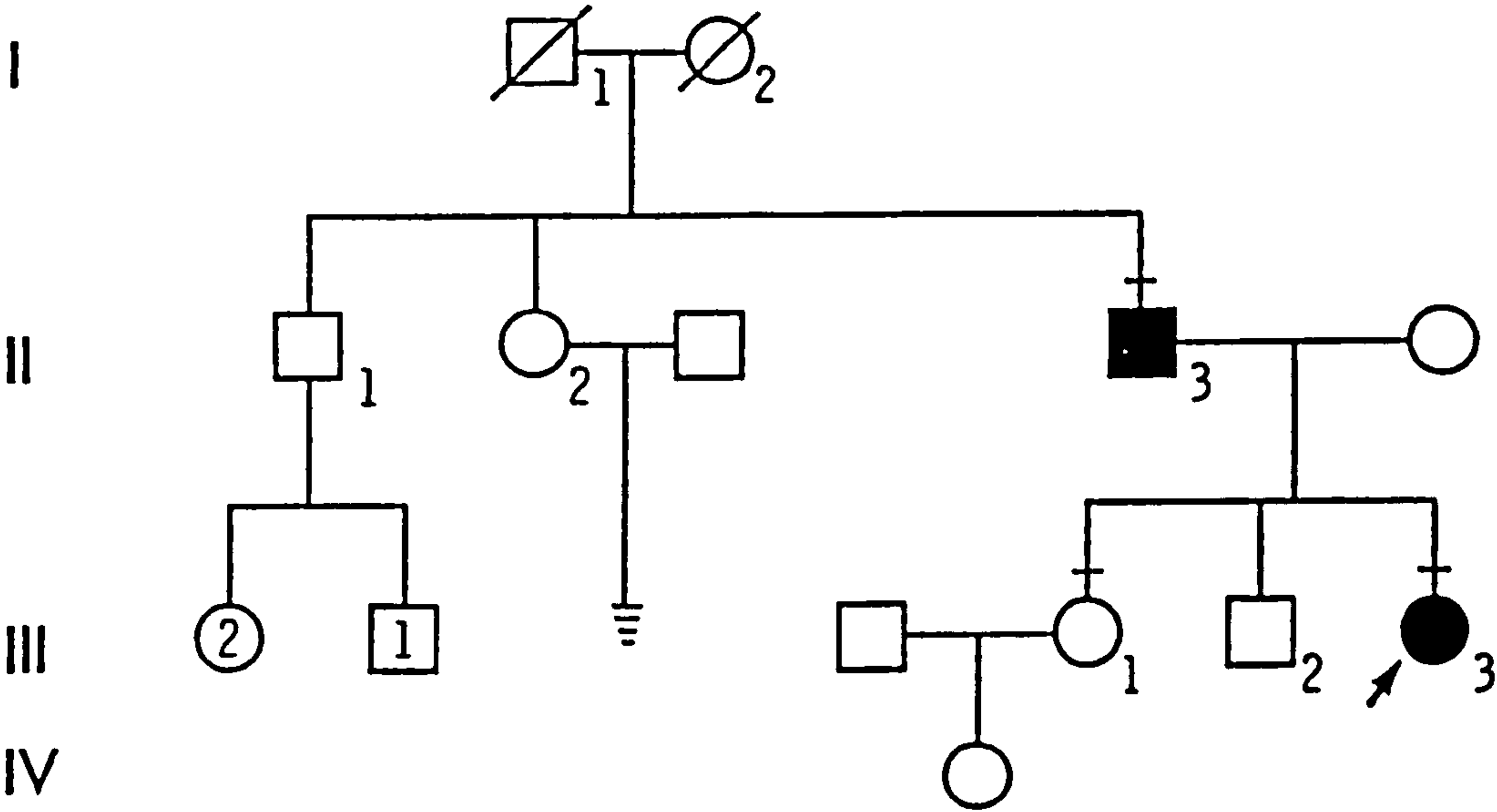
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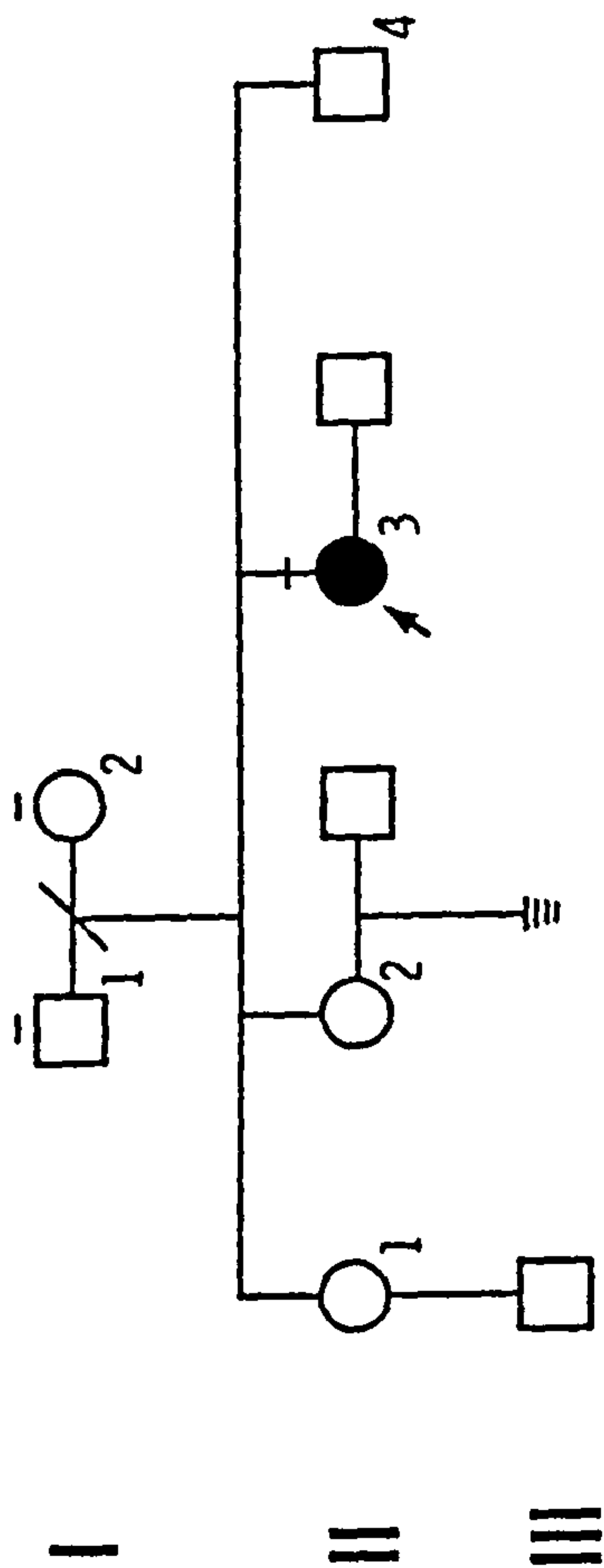
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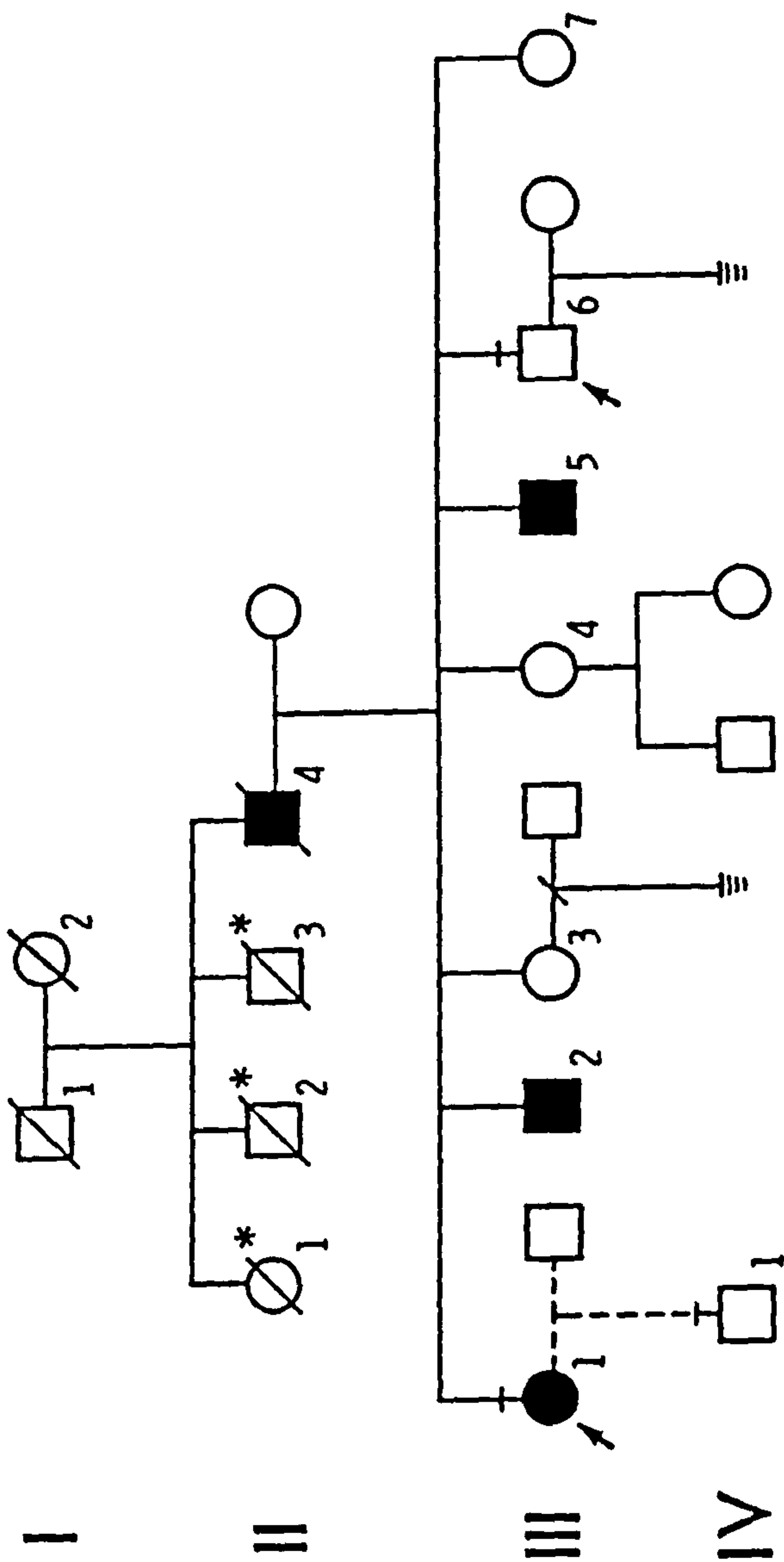
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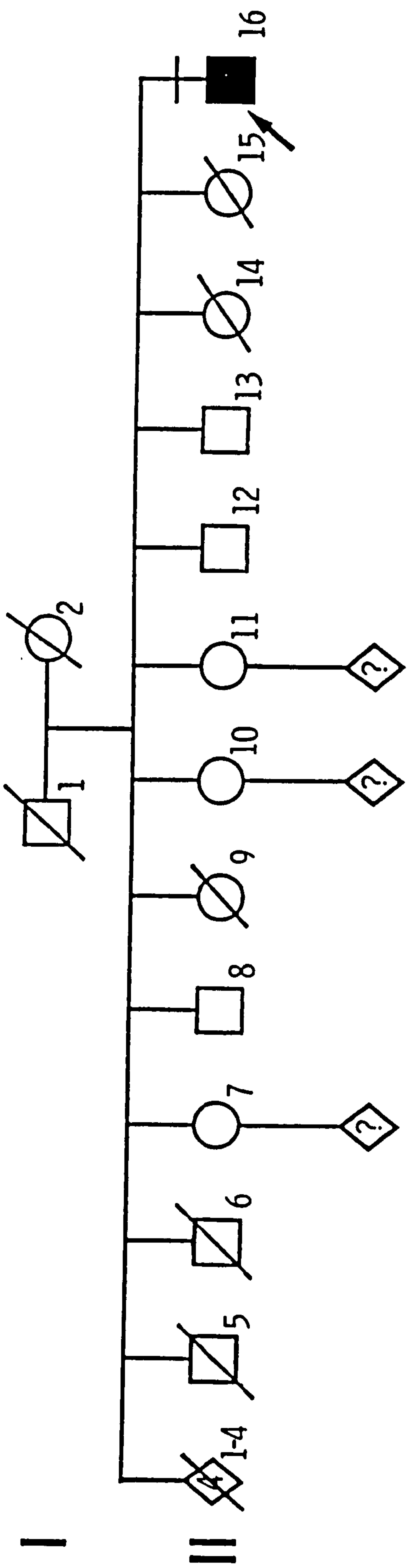
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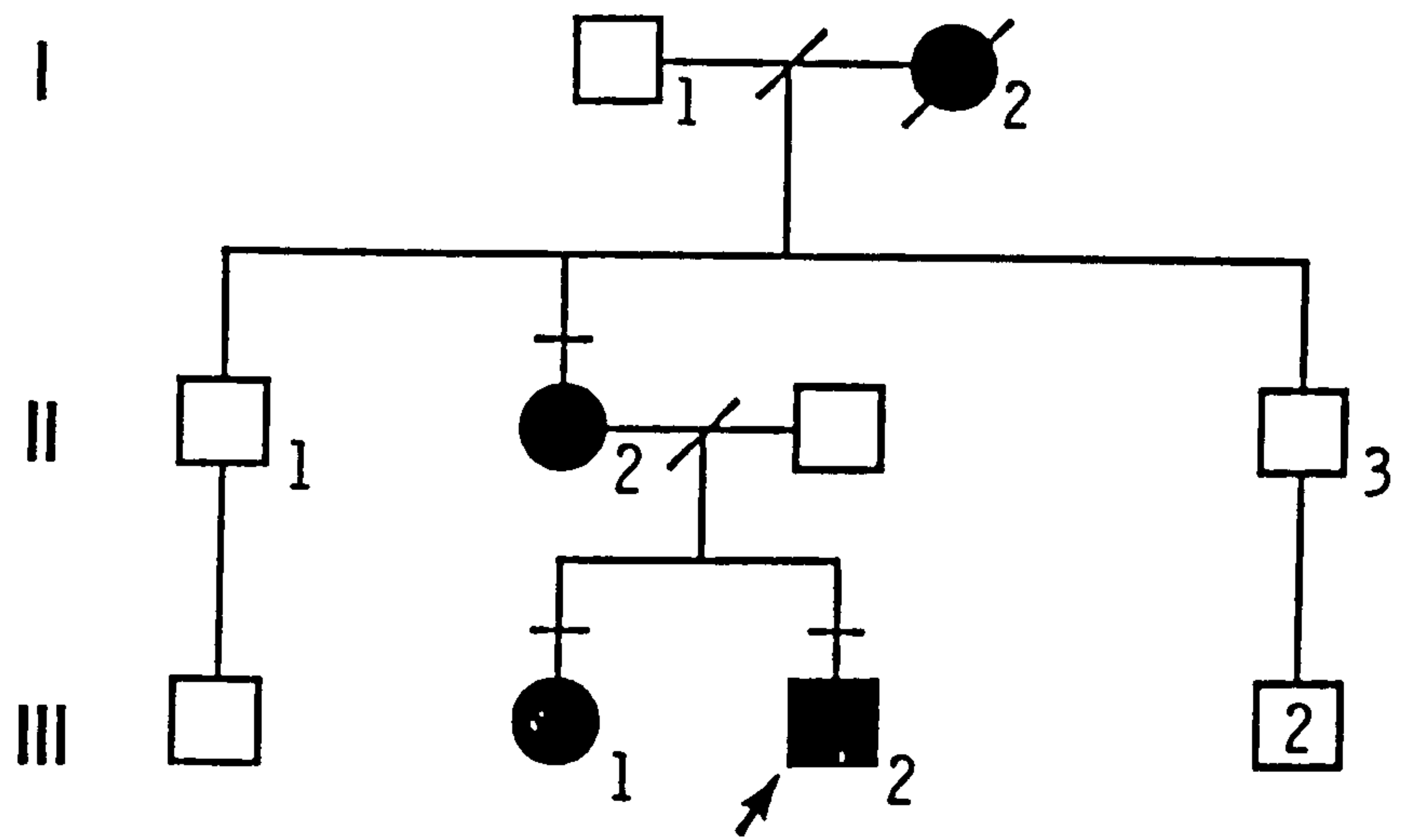
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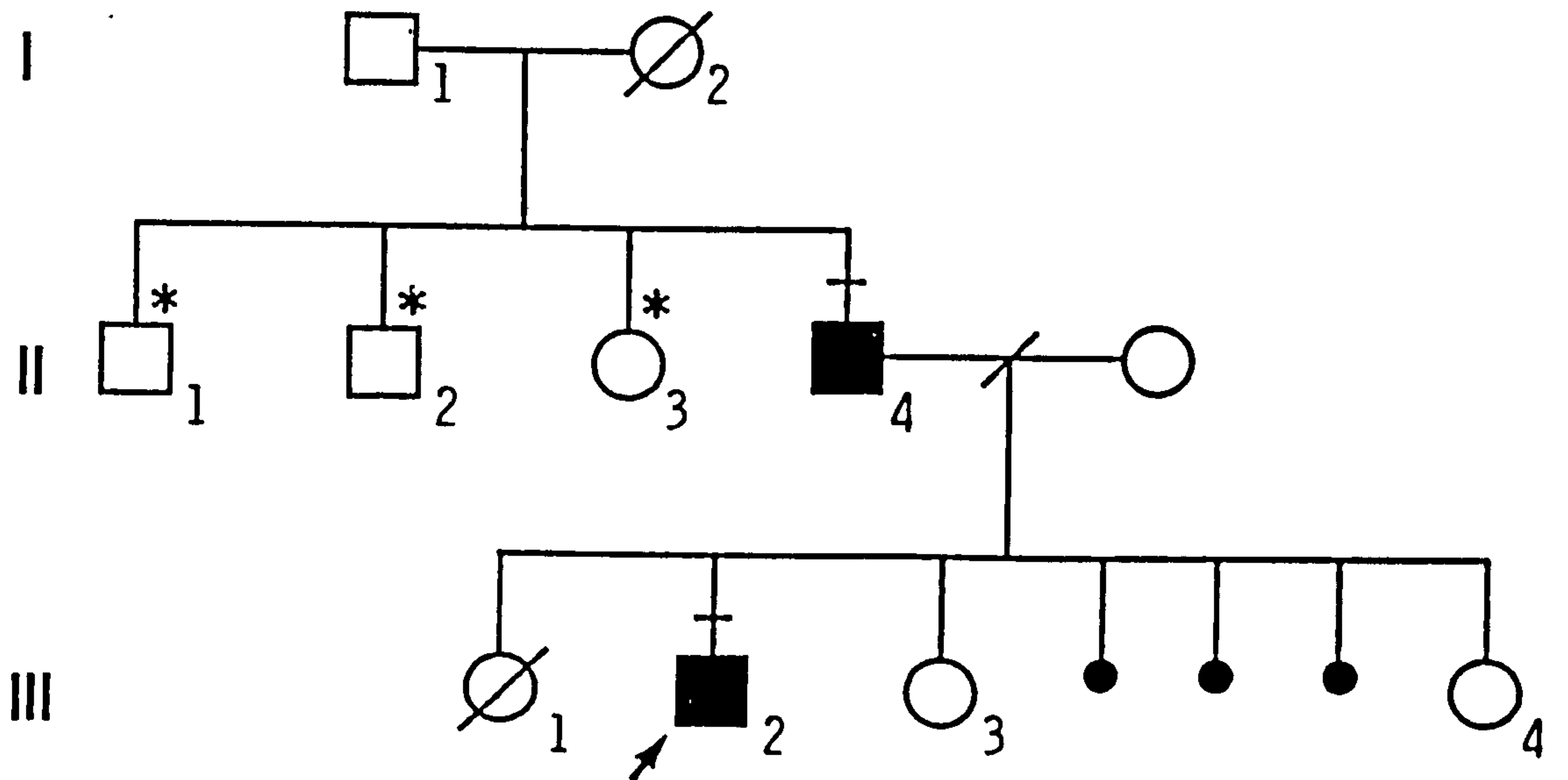
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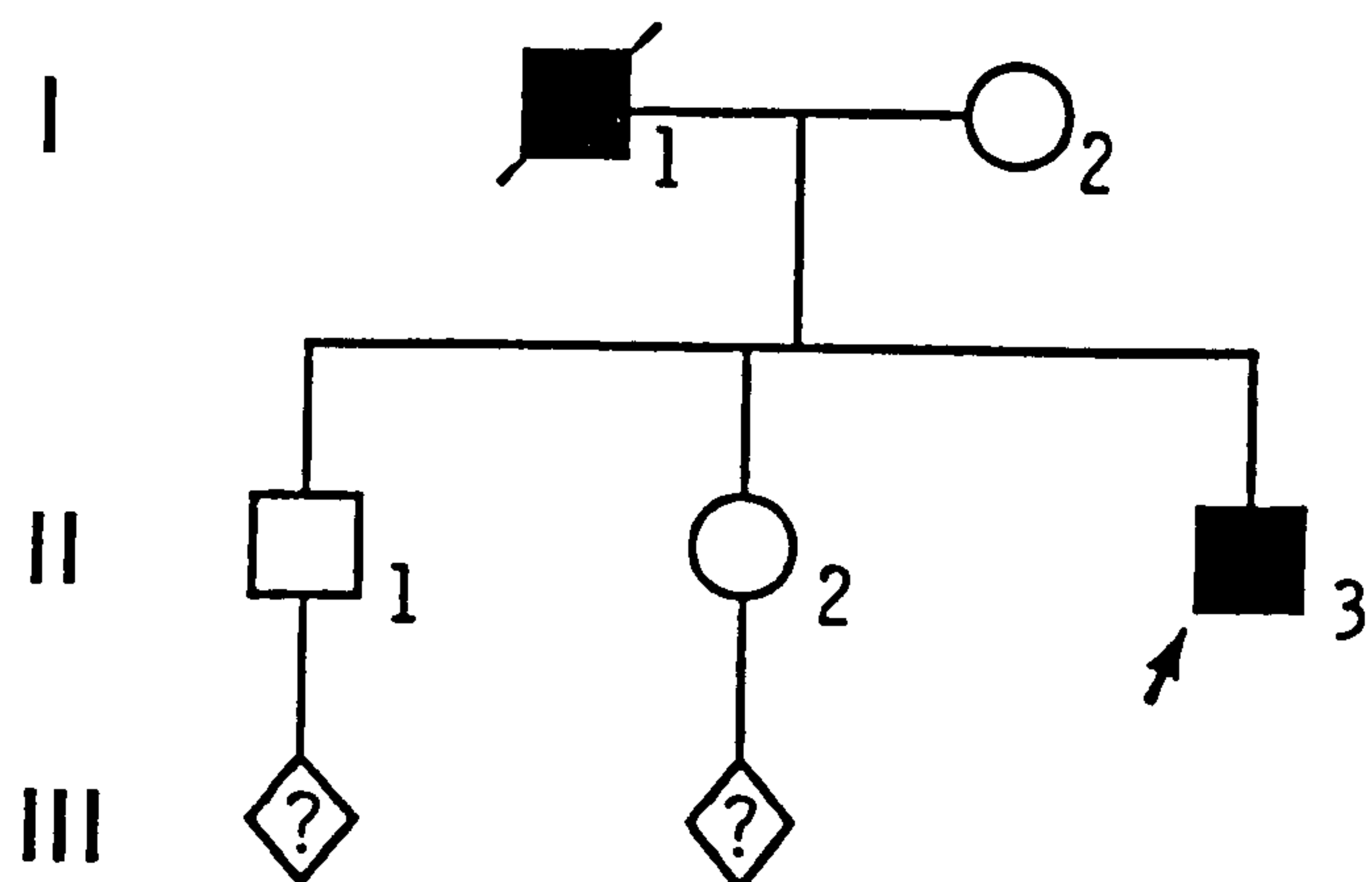
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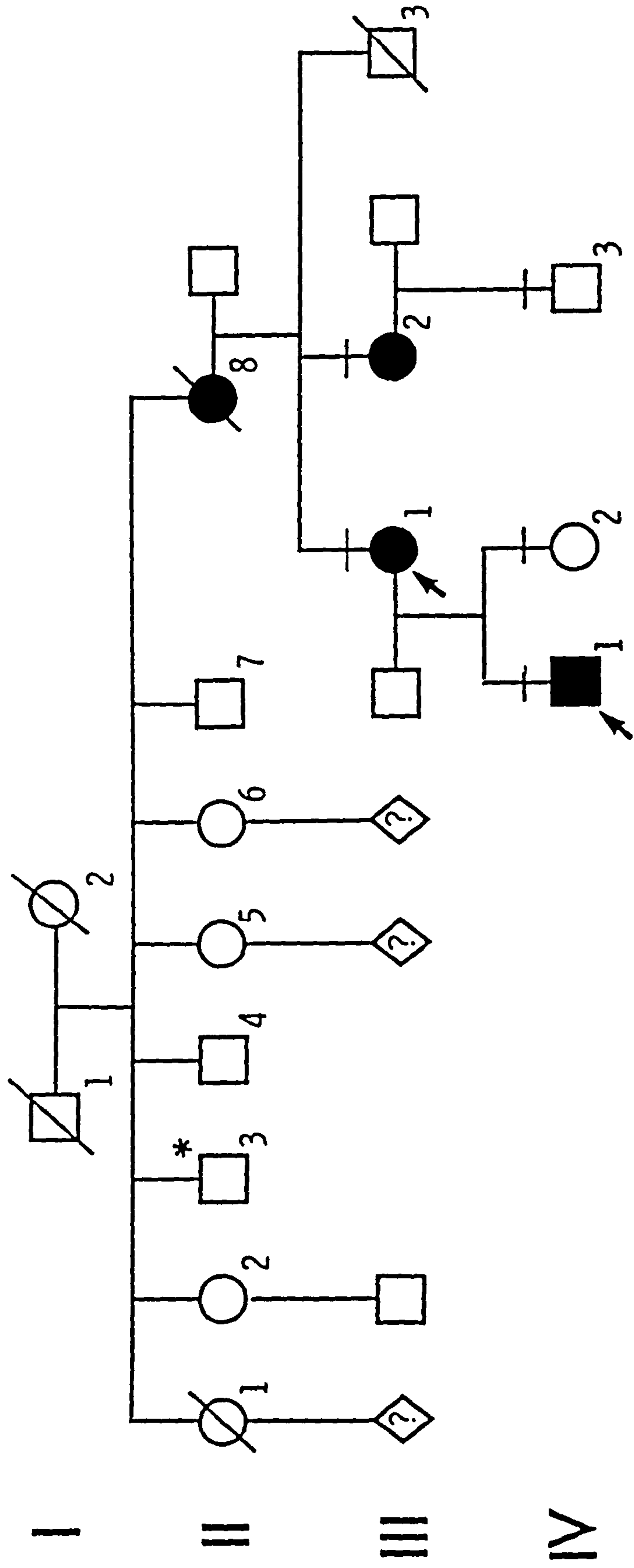
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NF40

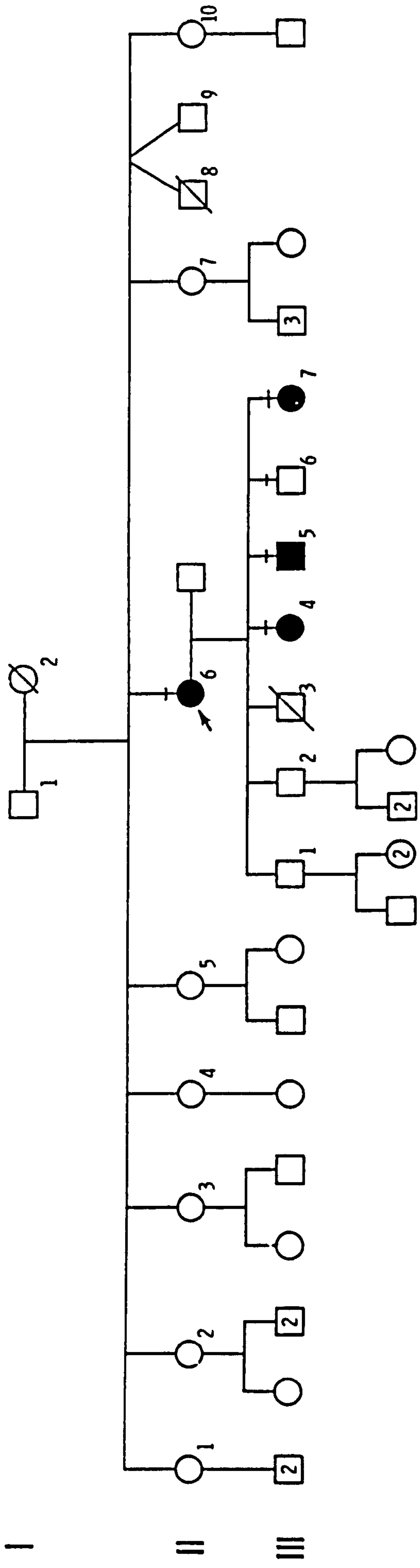


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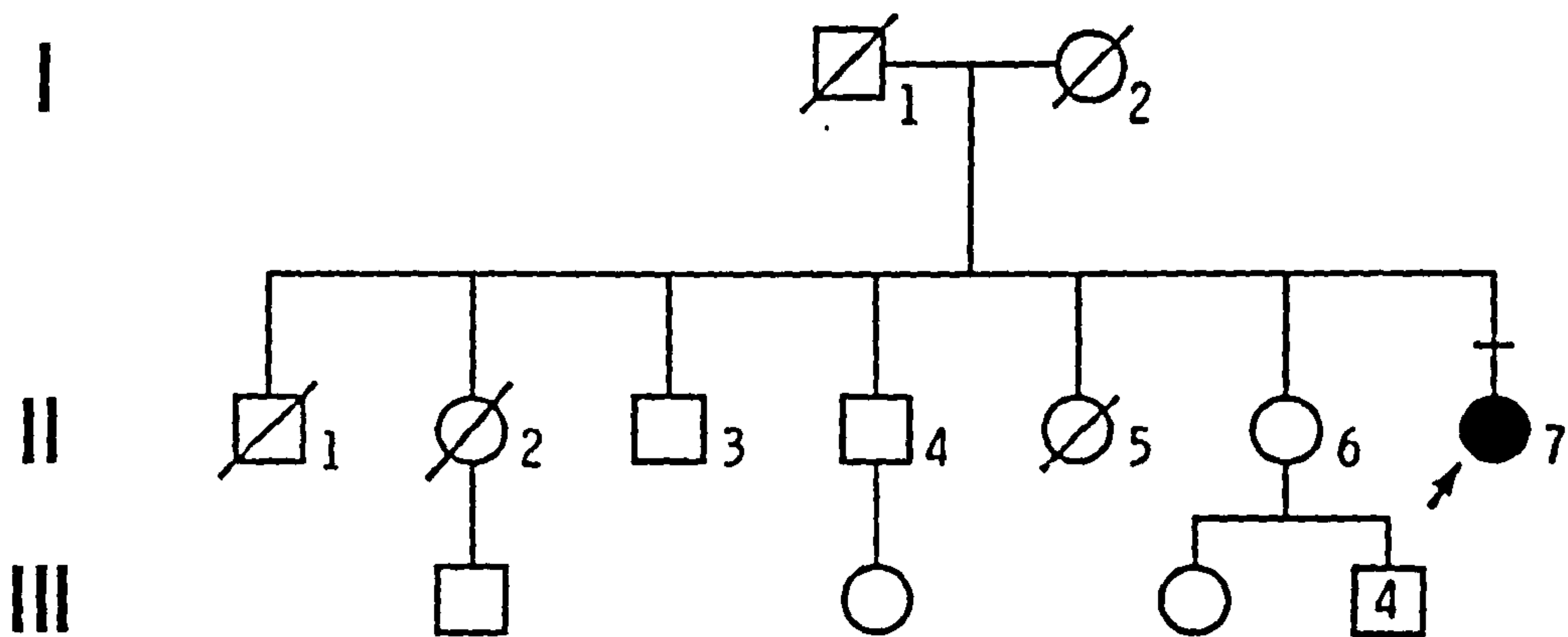


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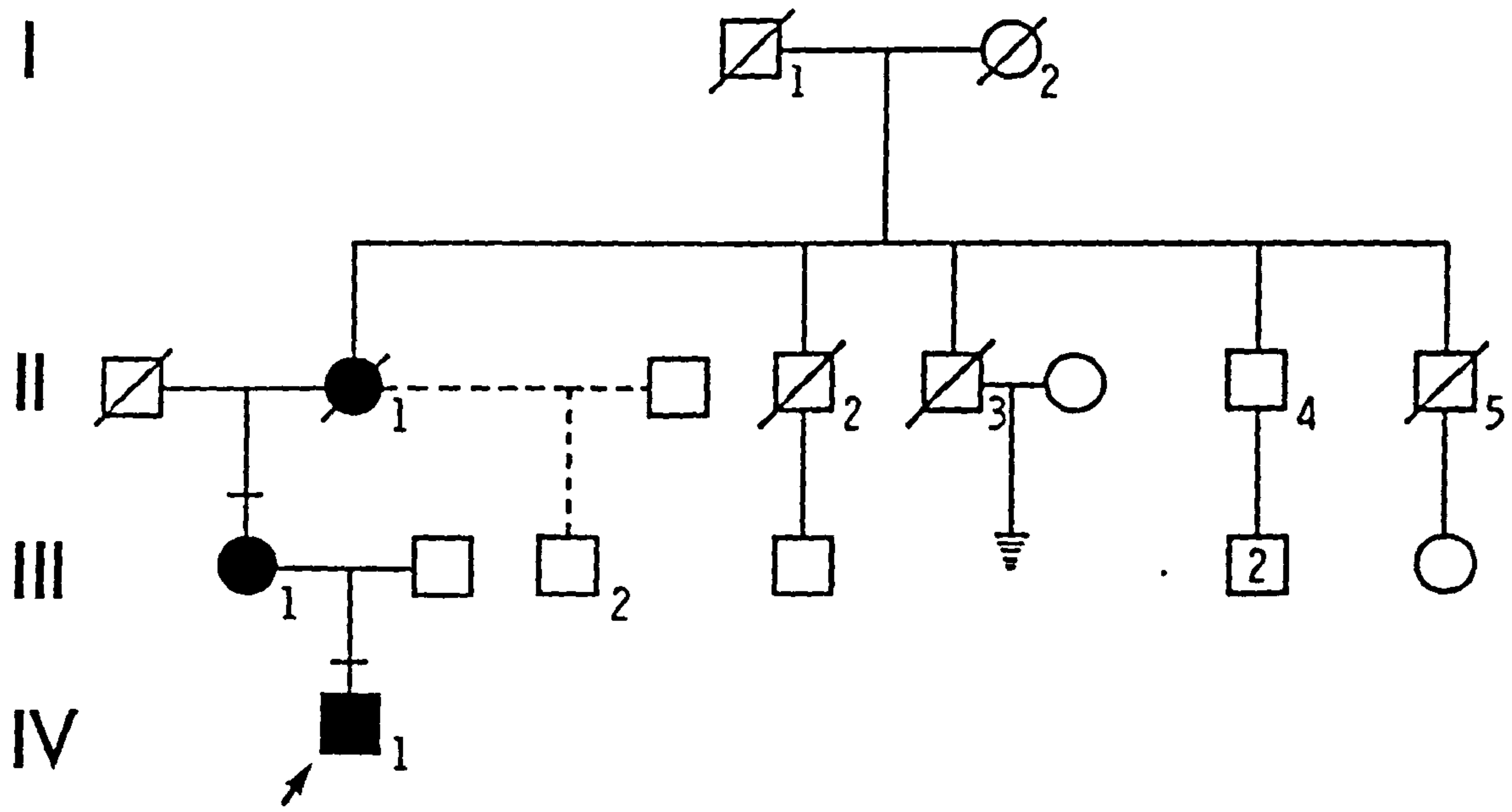
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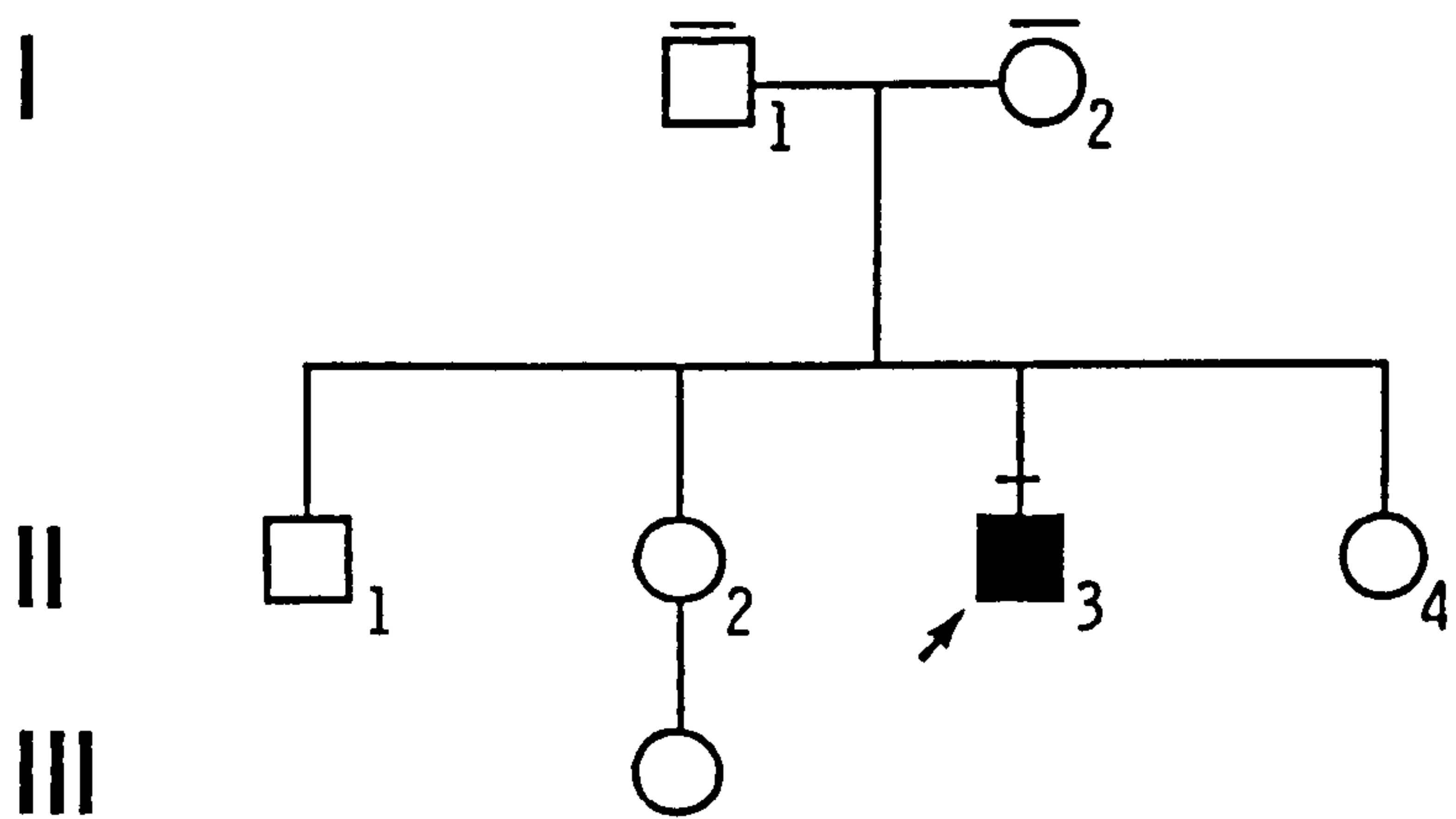
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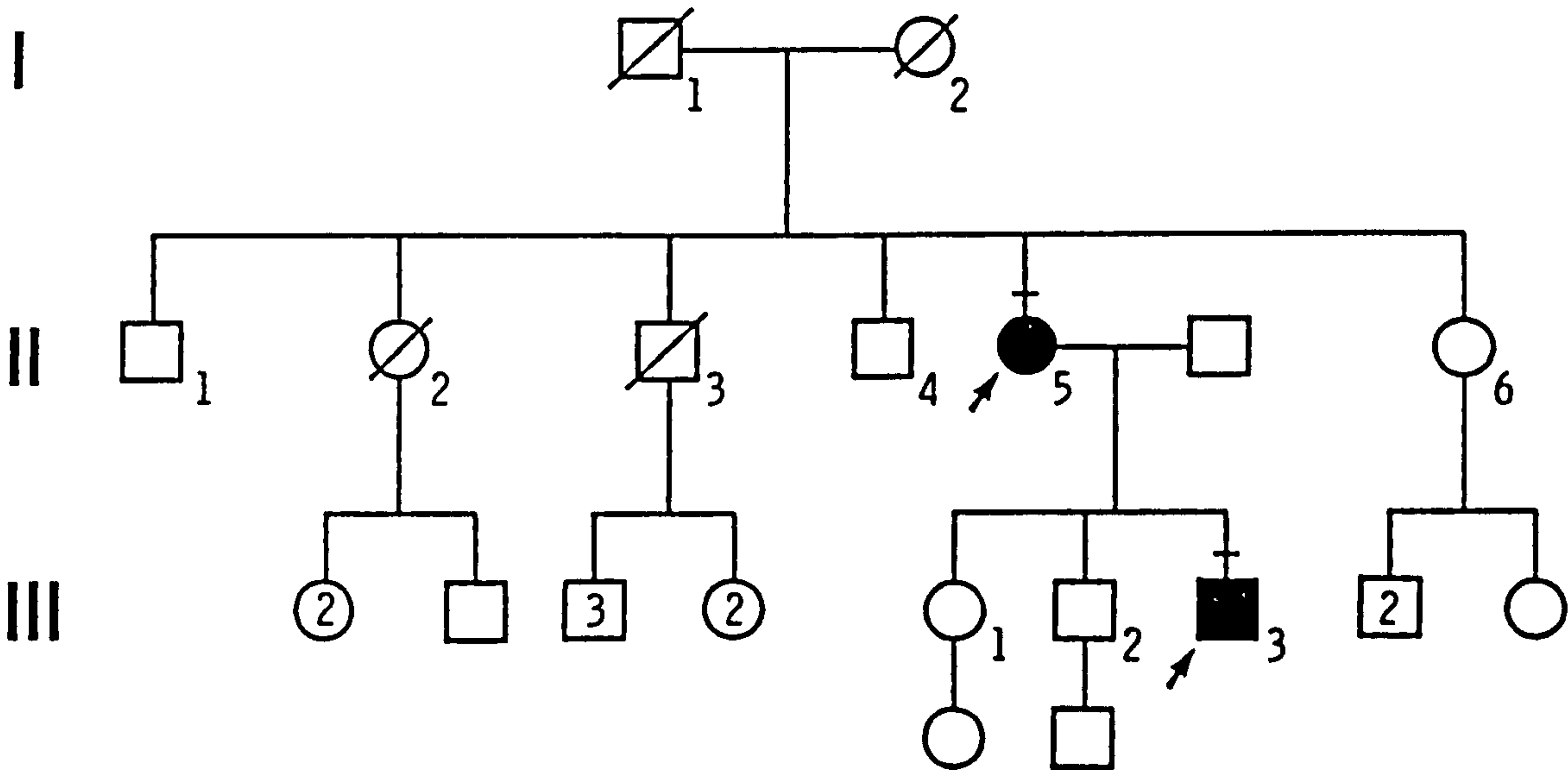
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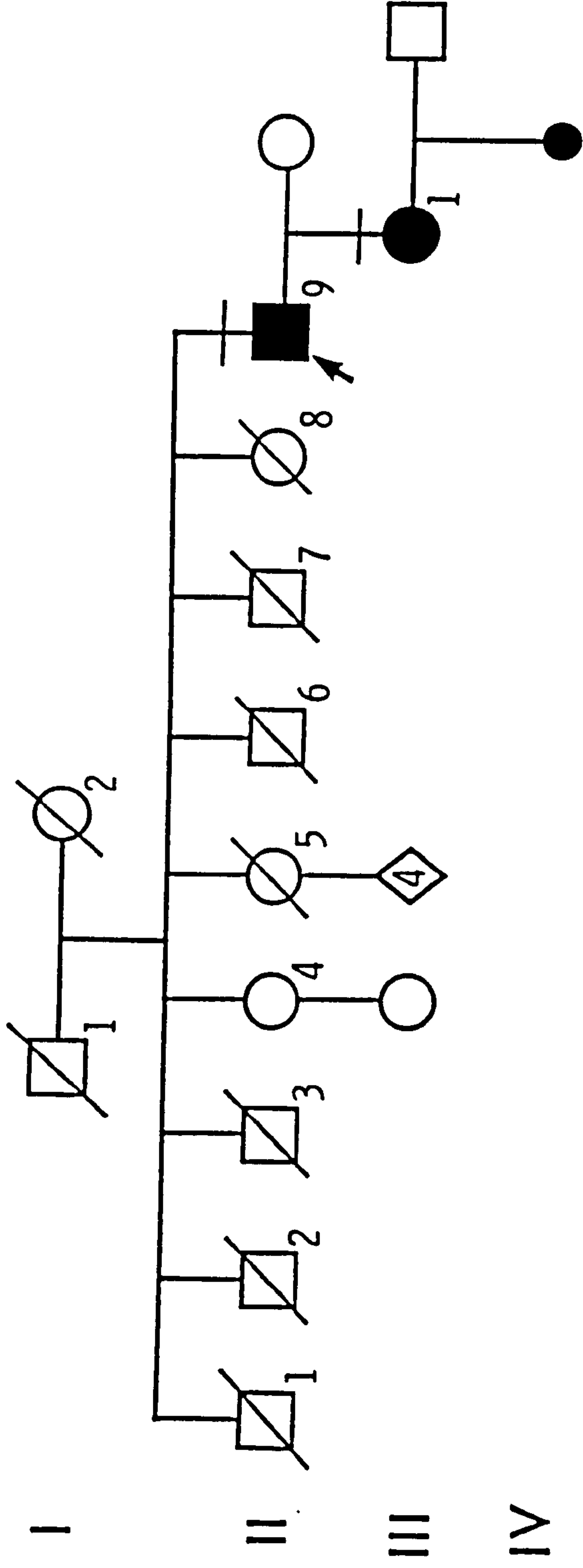
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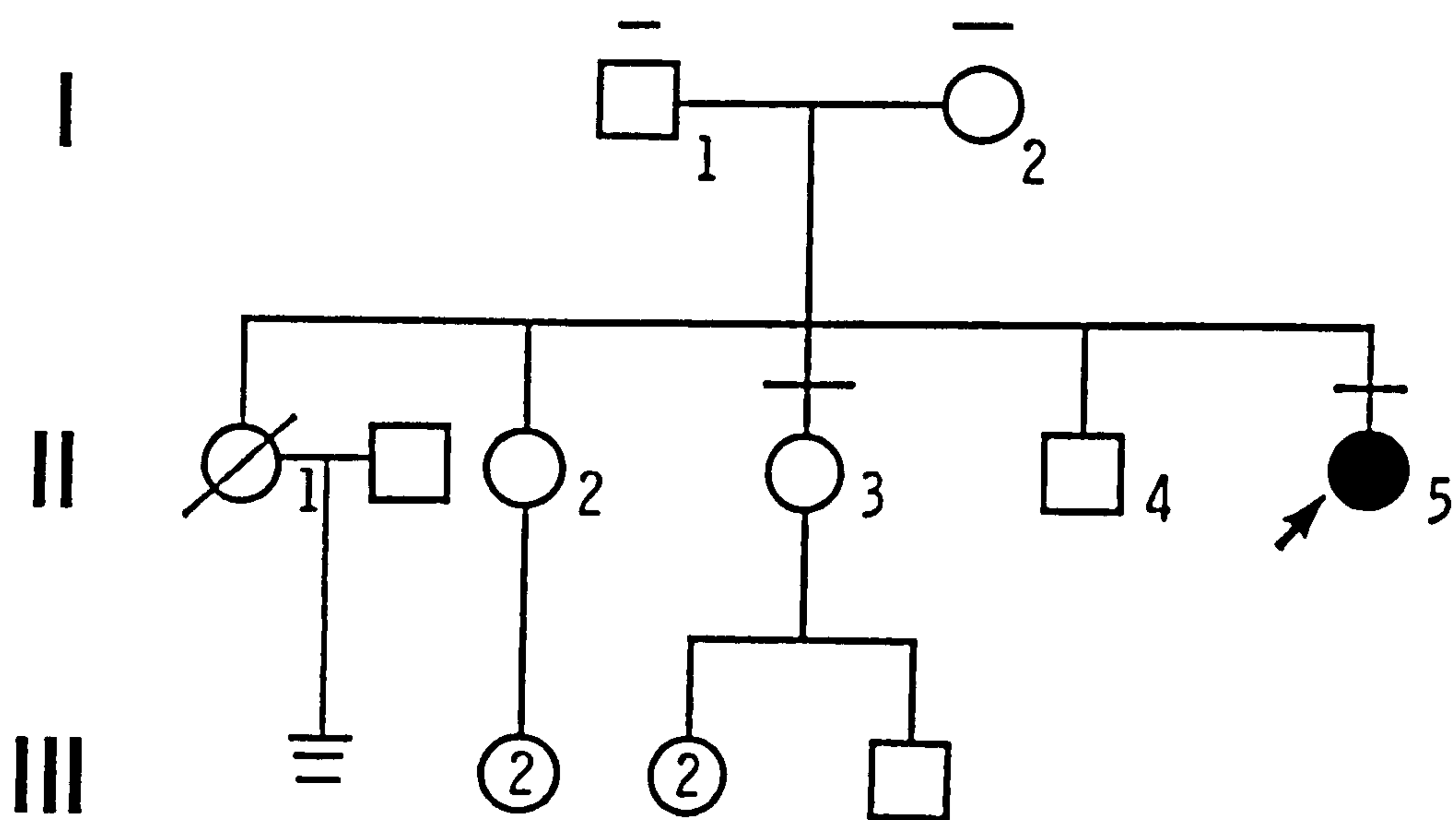
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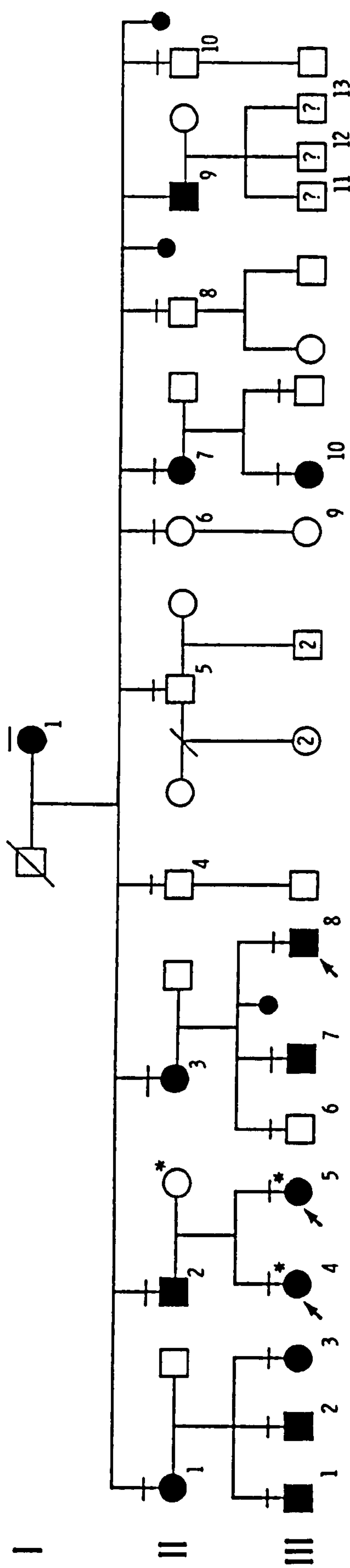
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NF48

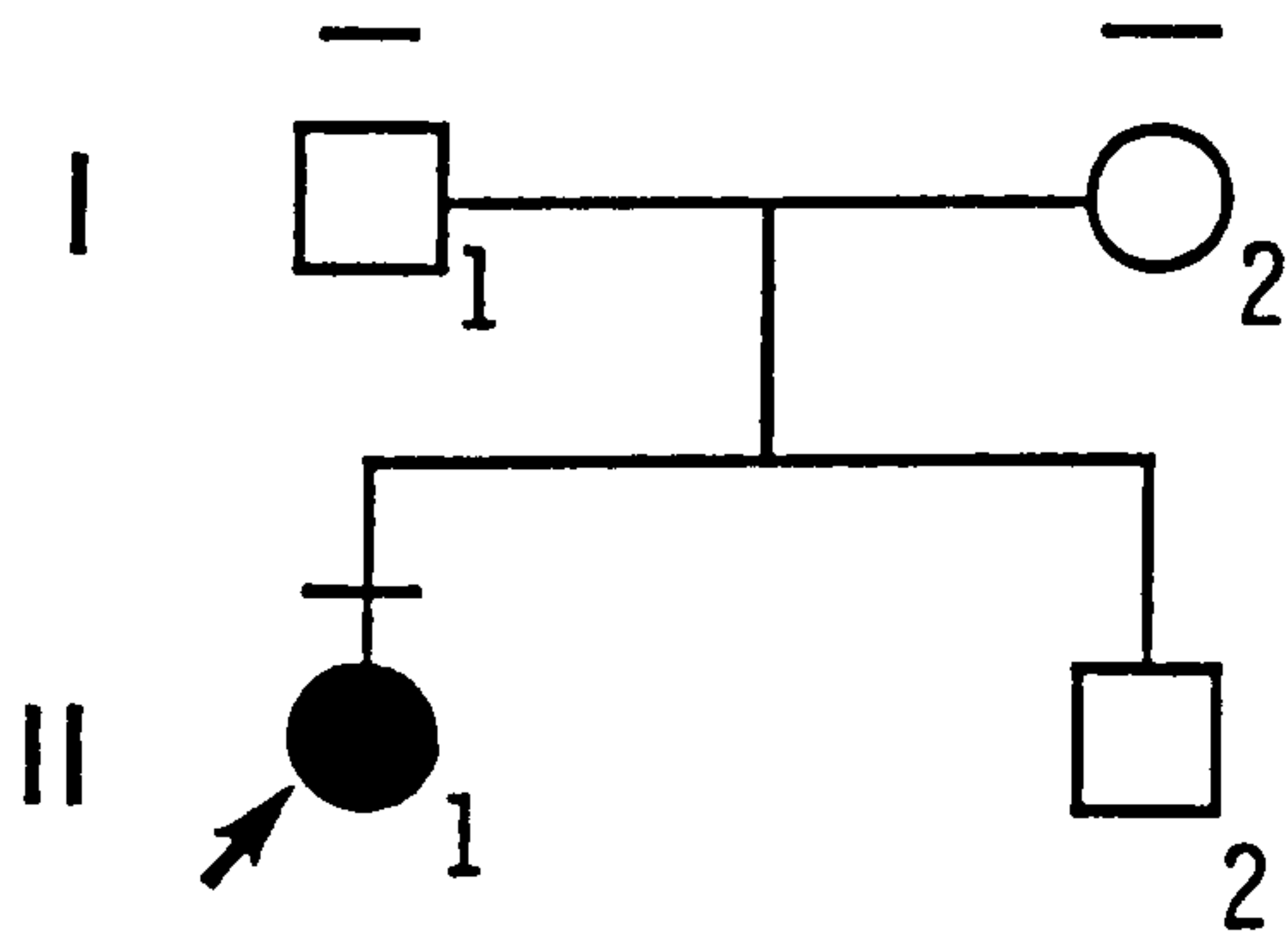


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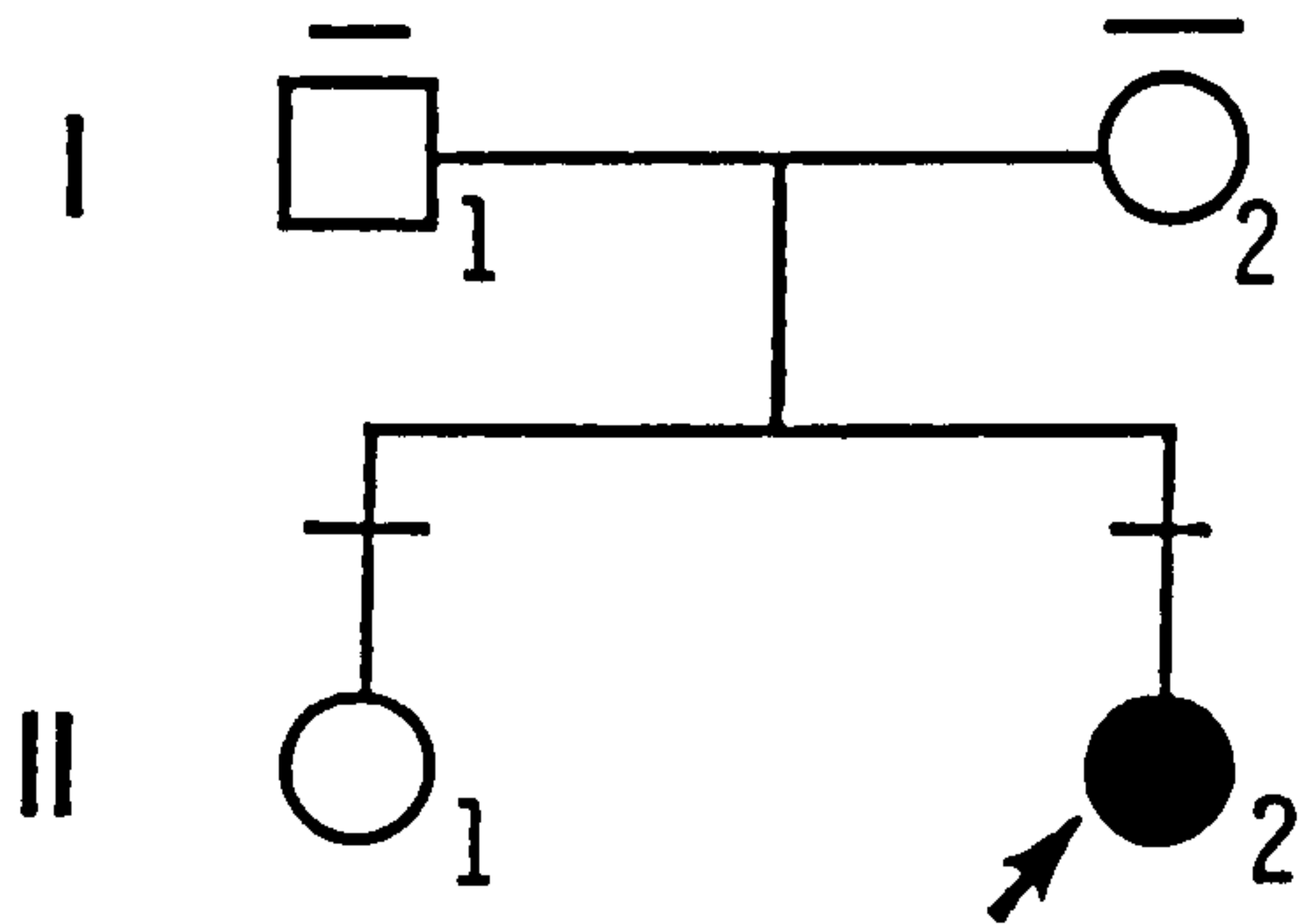


* Multiple epiphyseal
dysplasia

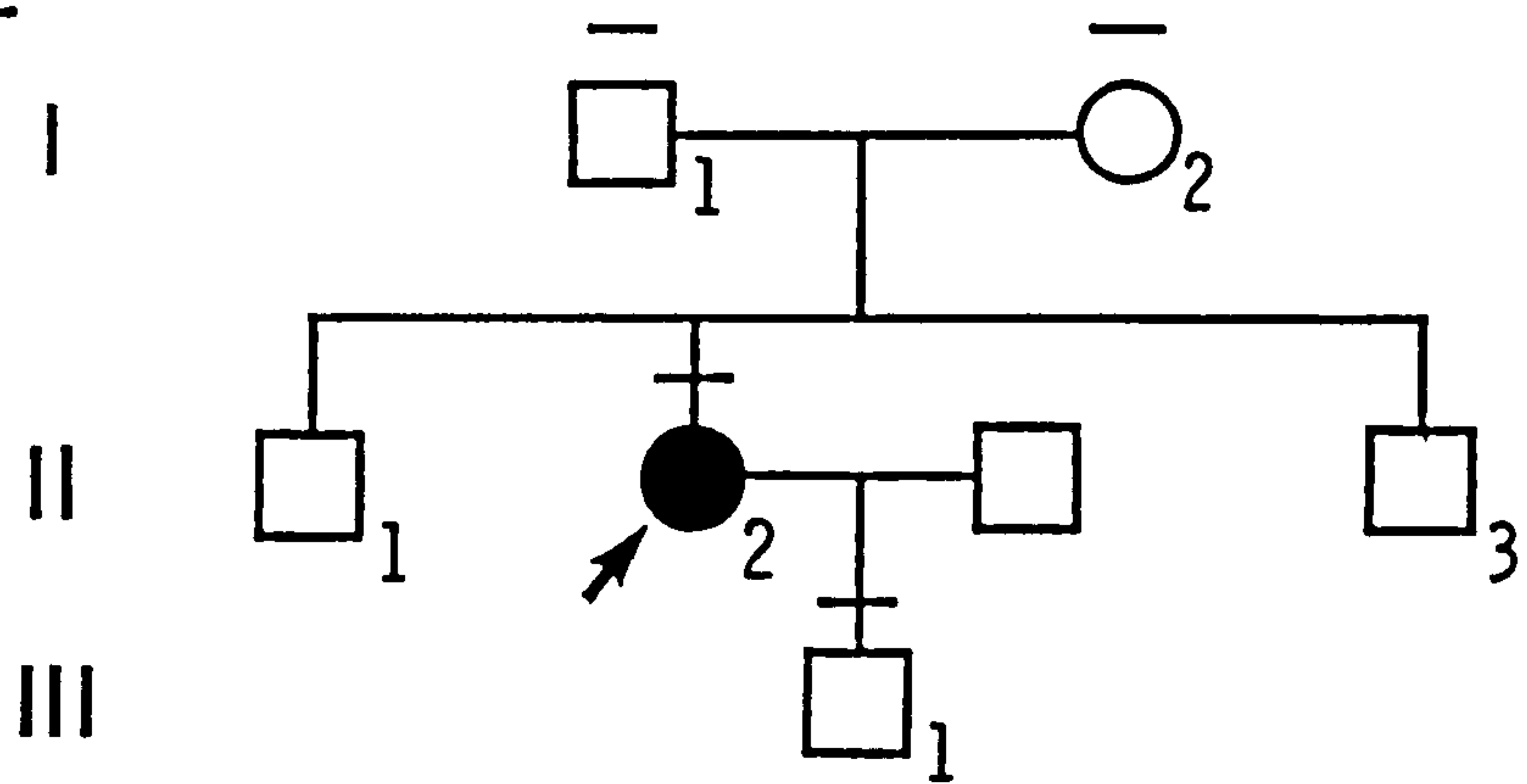
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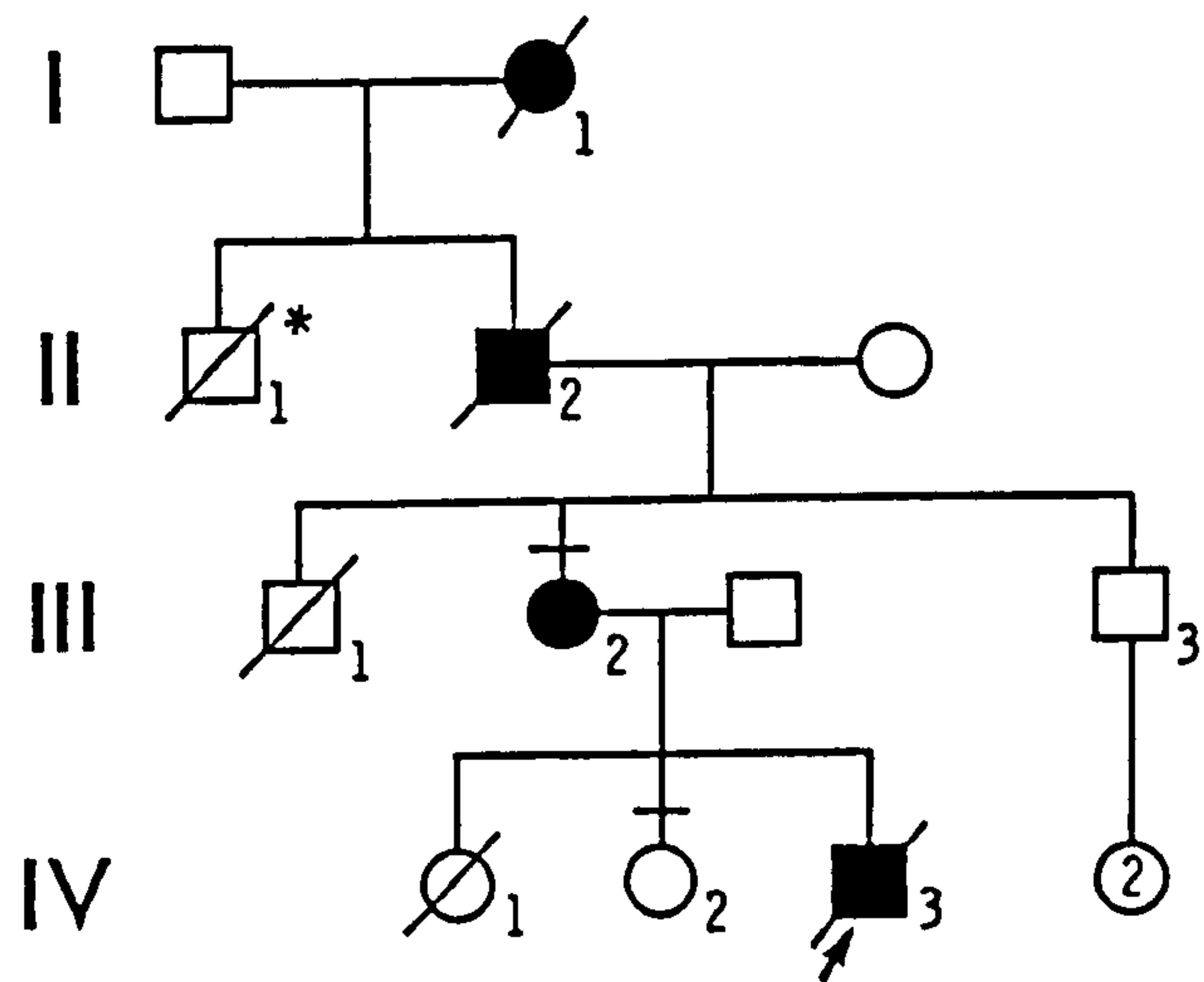
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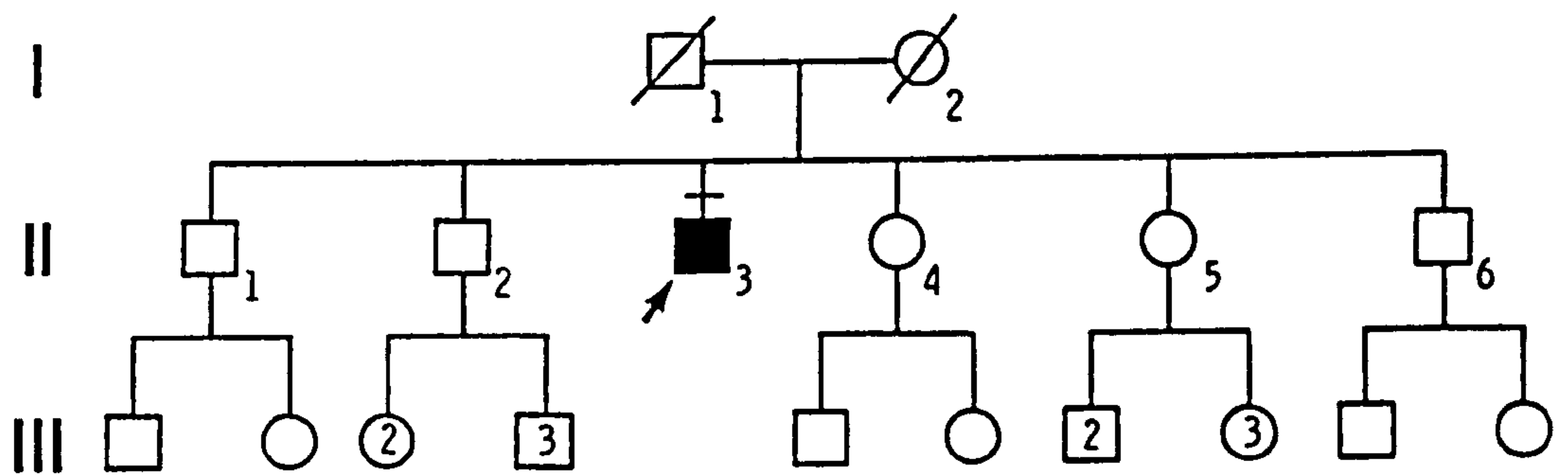
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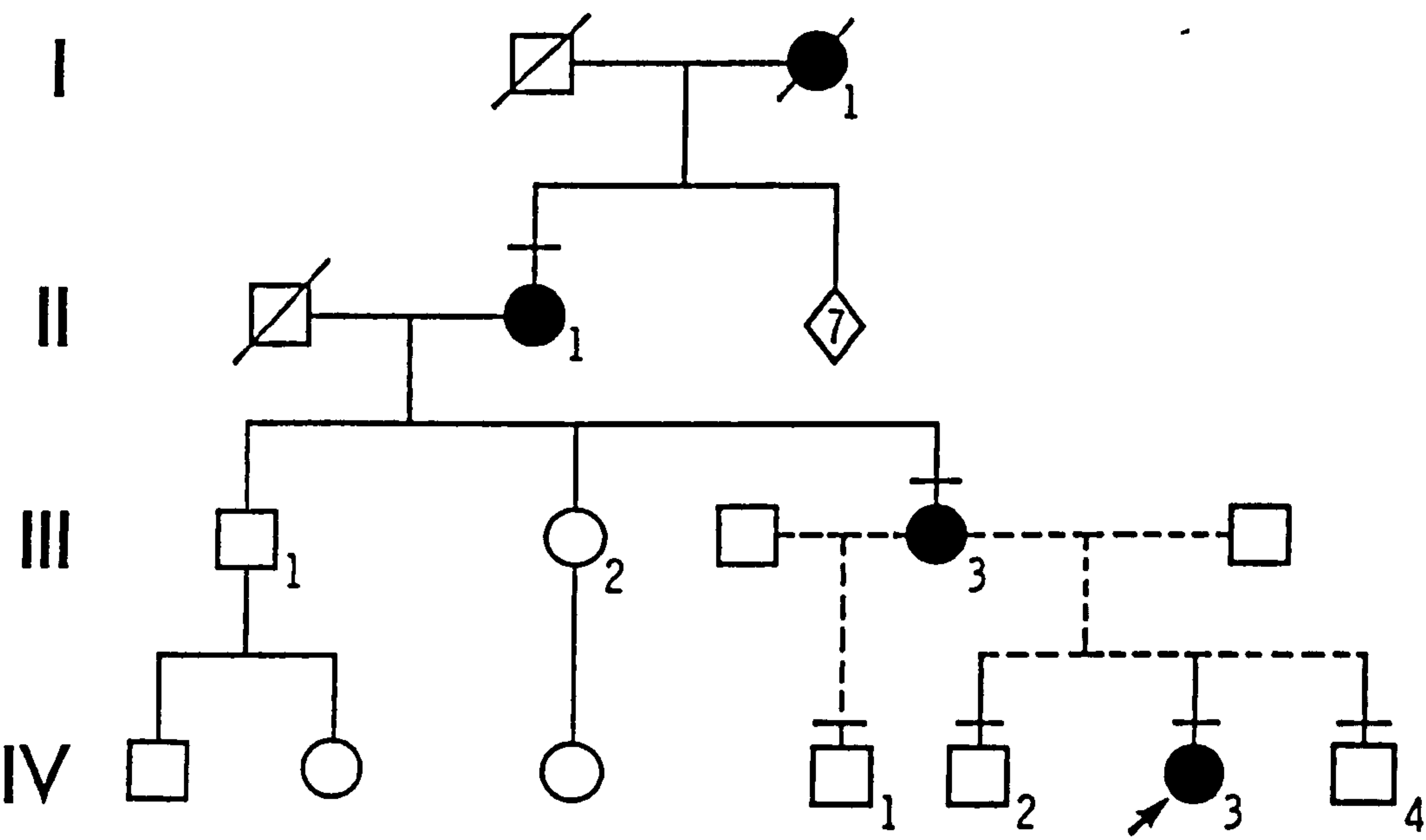
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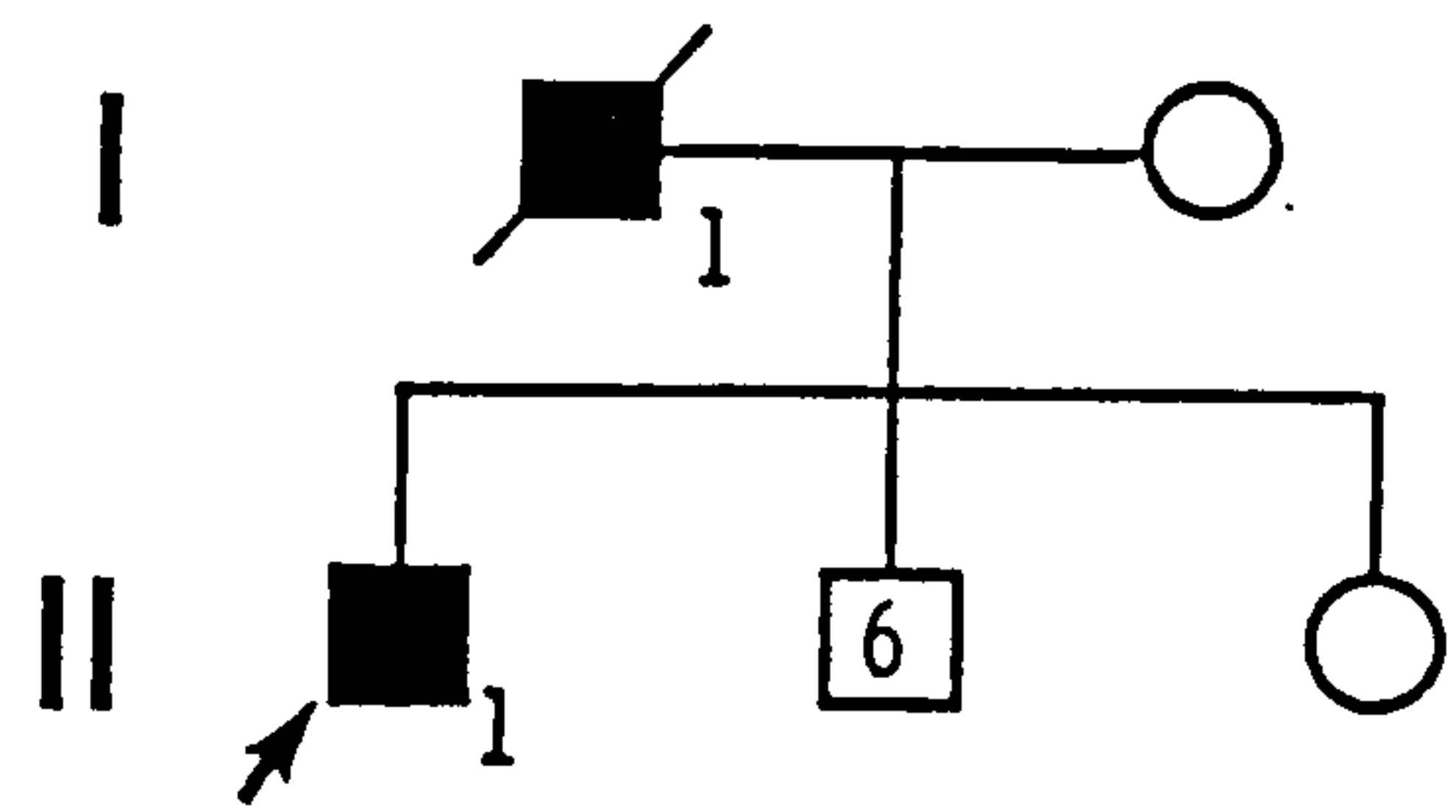
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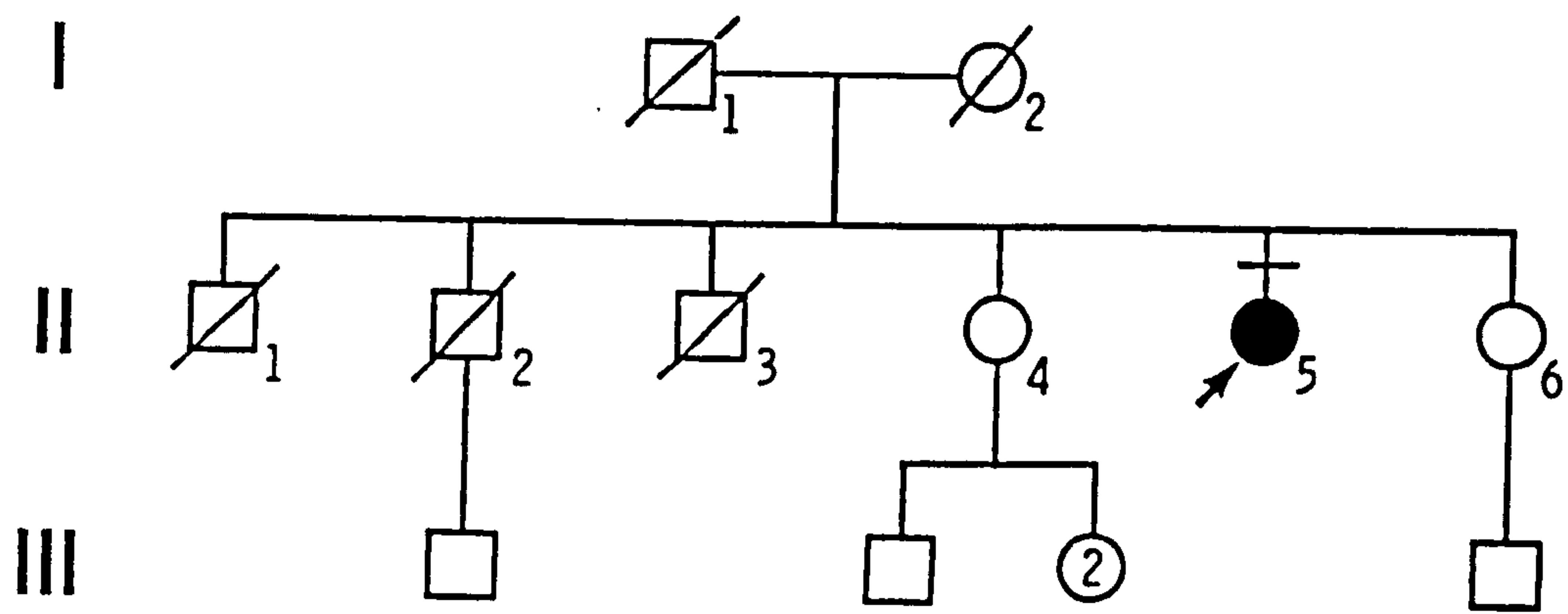
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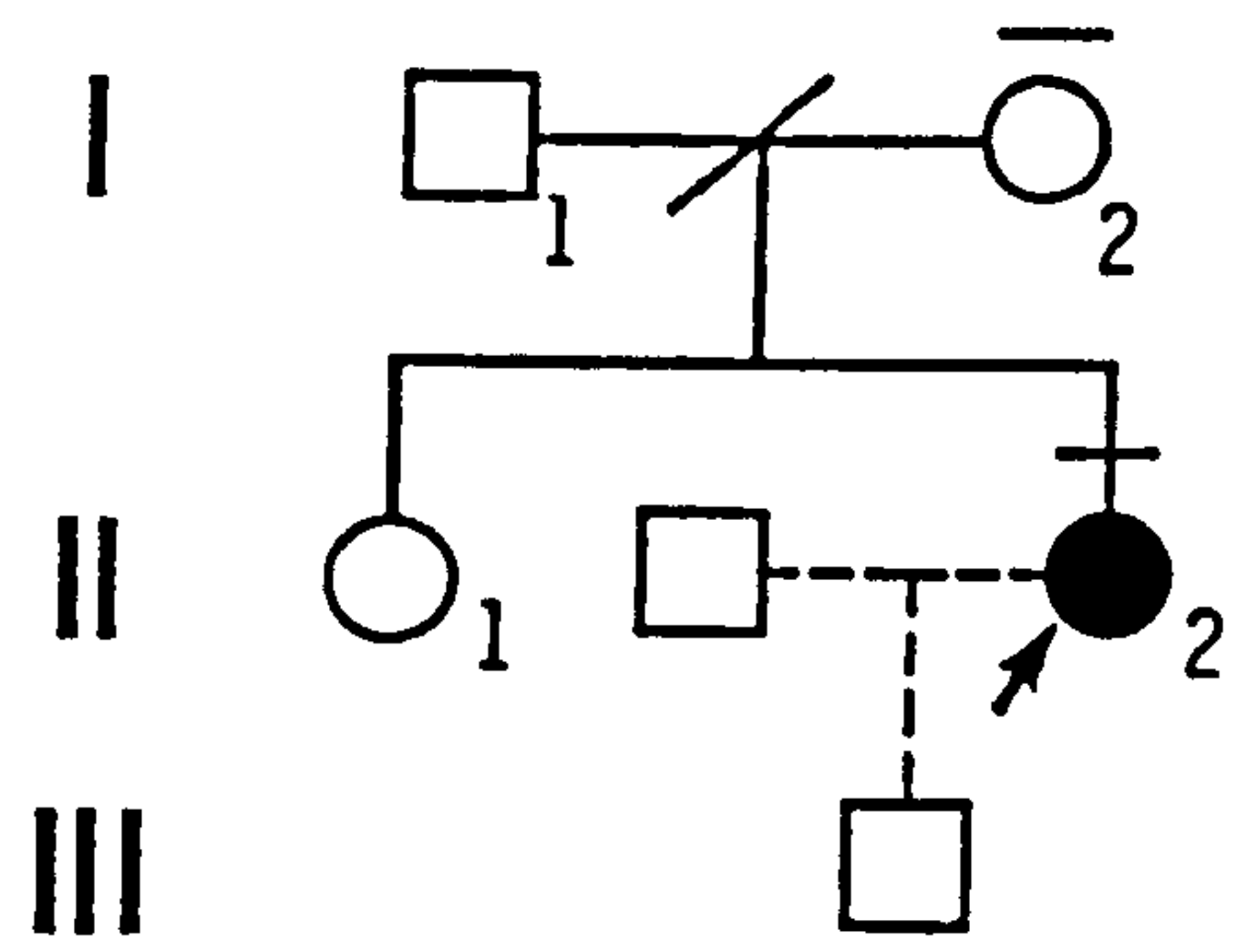
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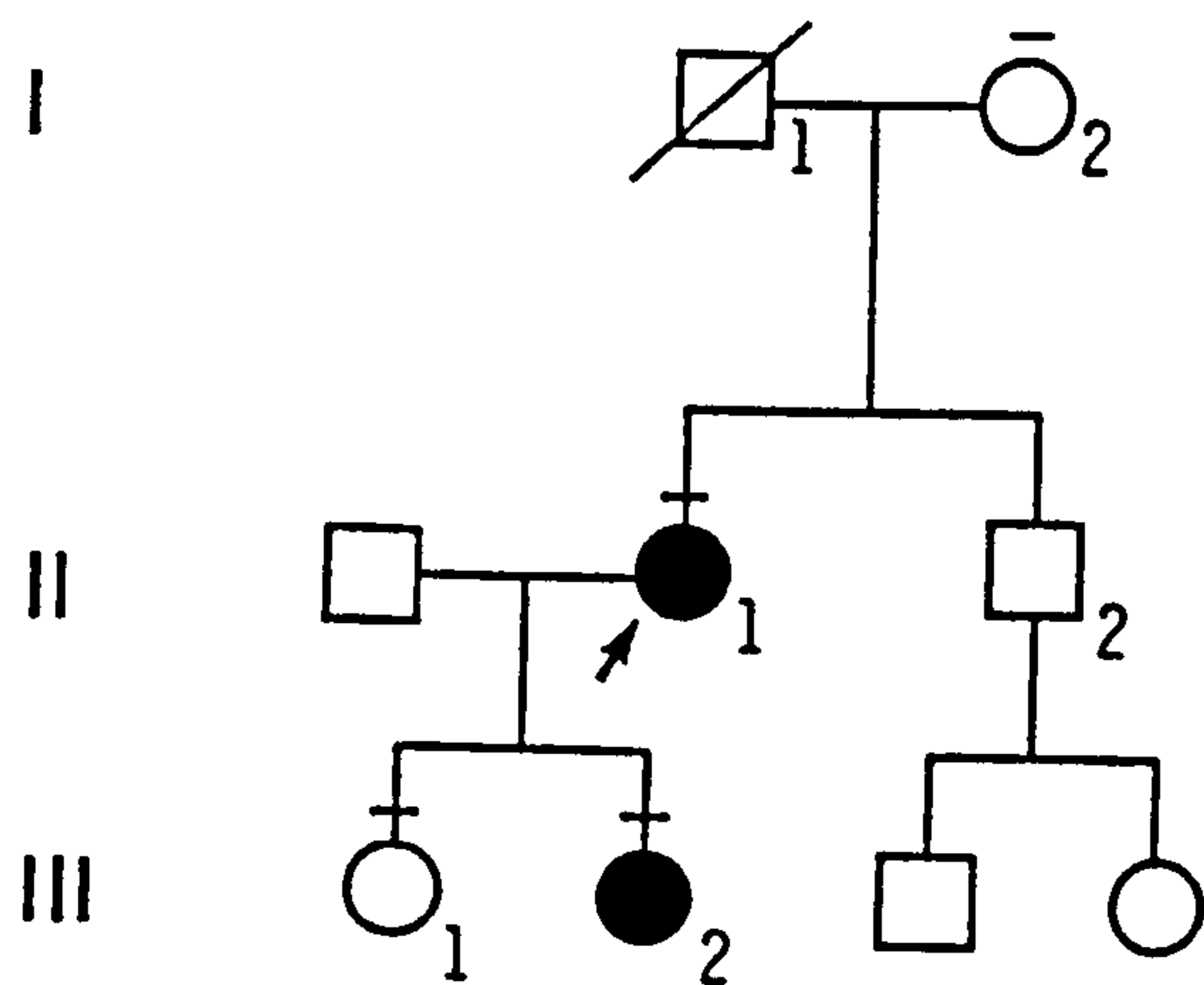
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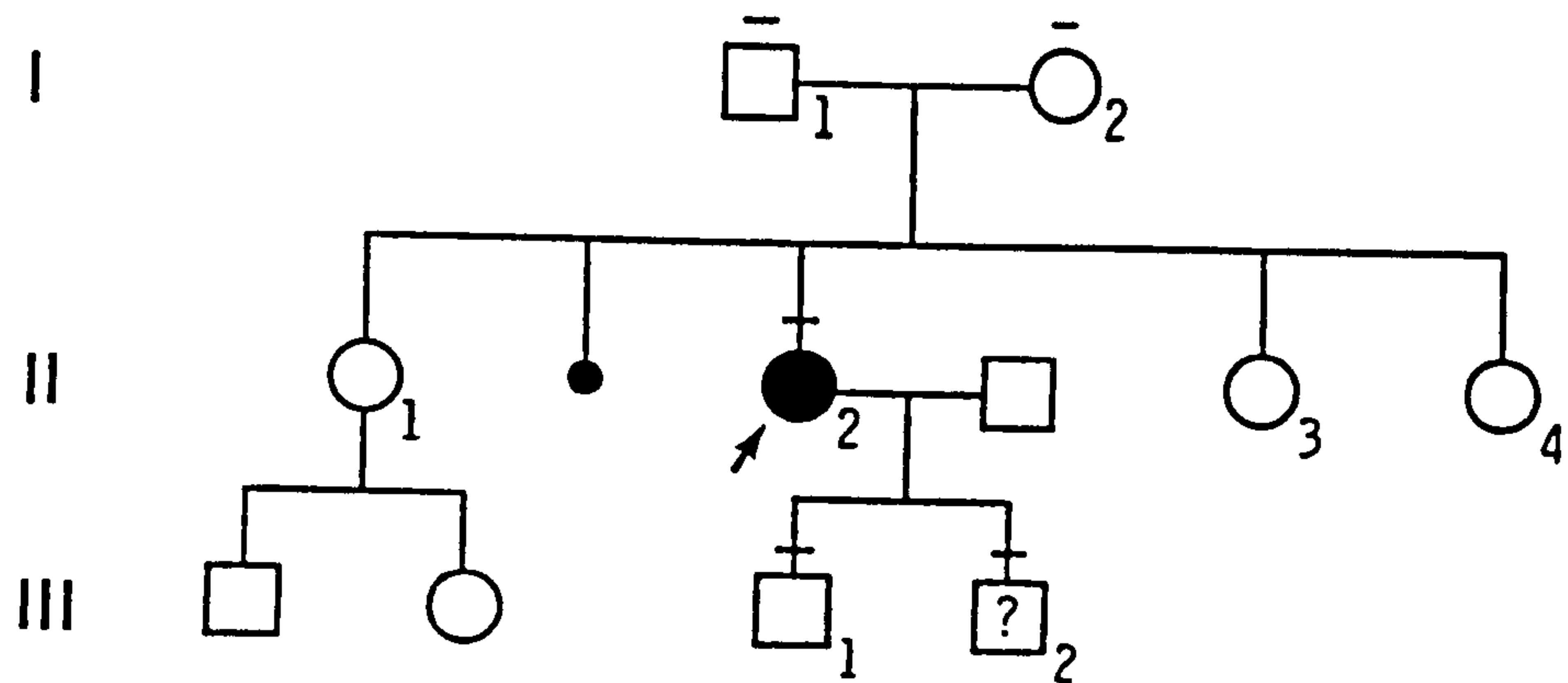
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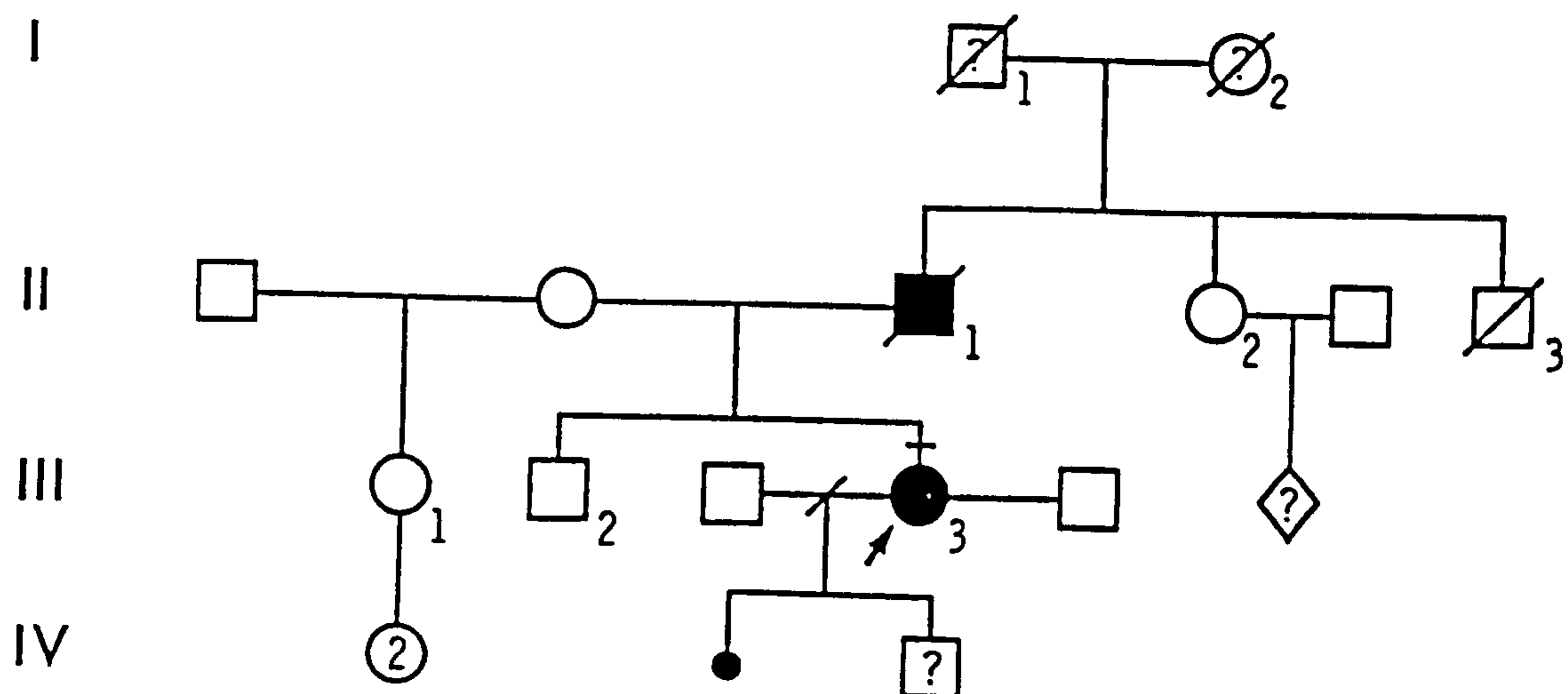
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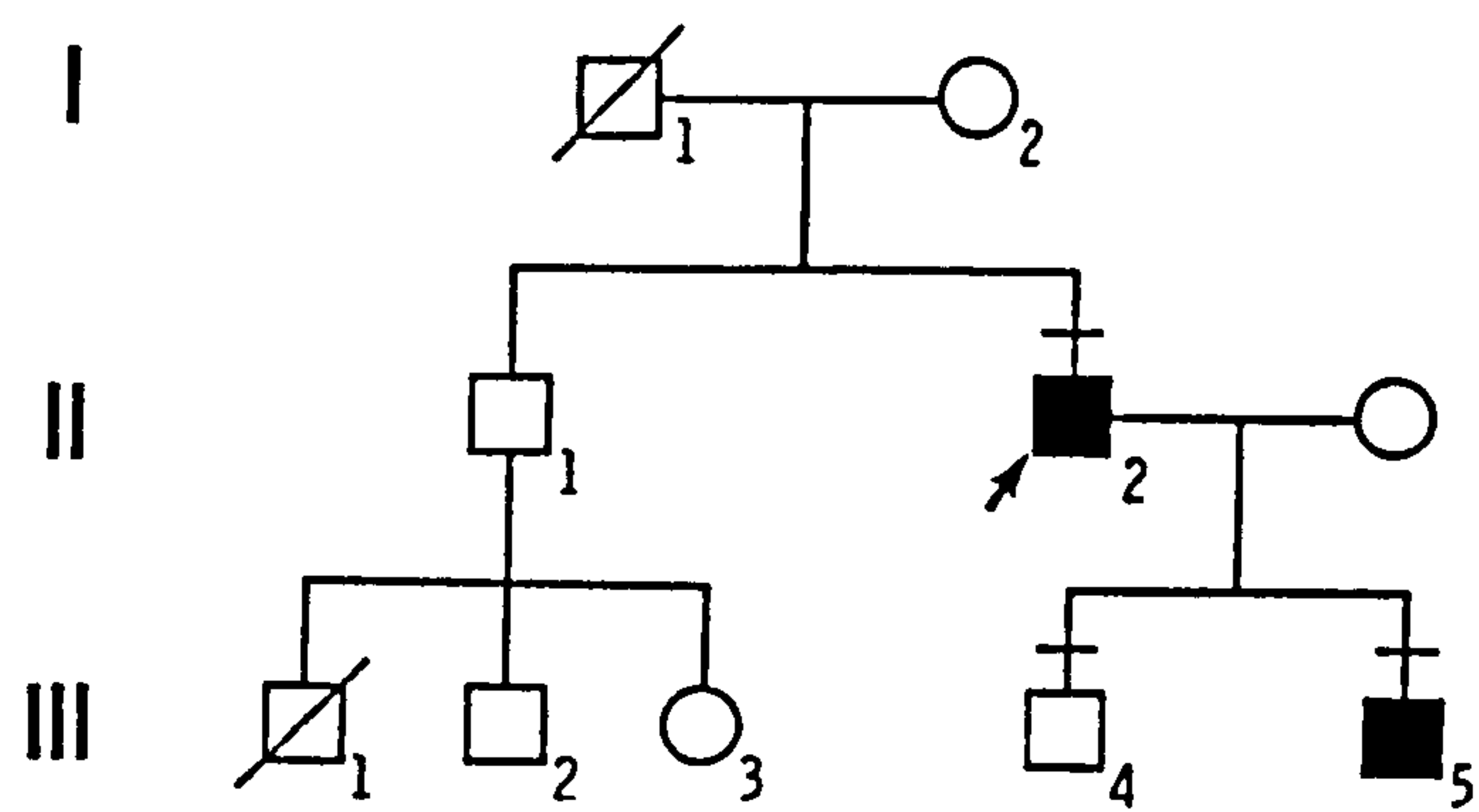
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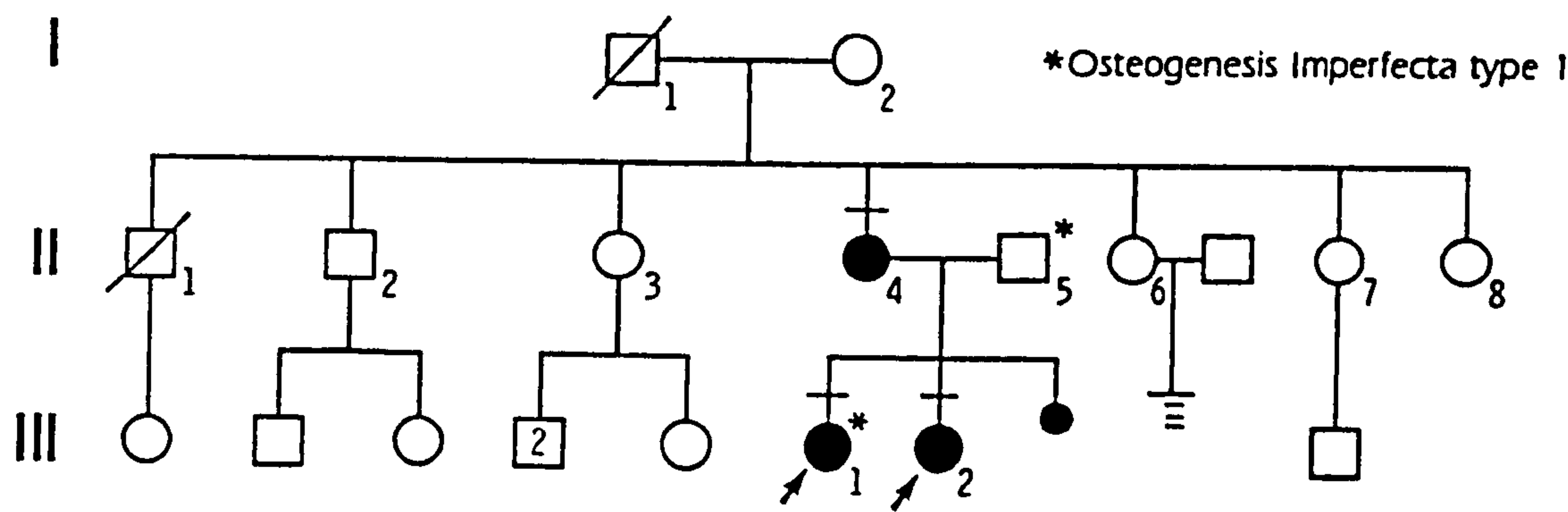
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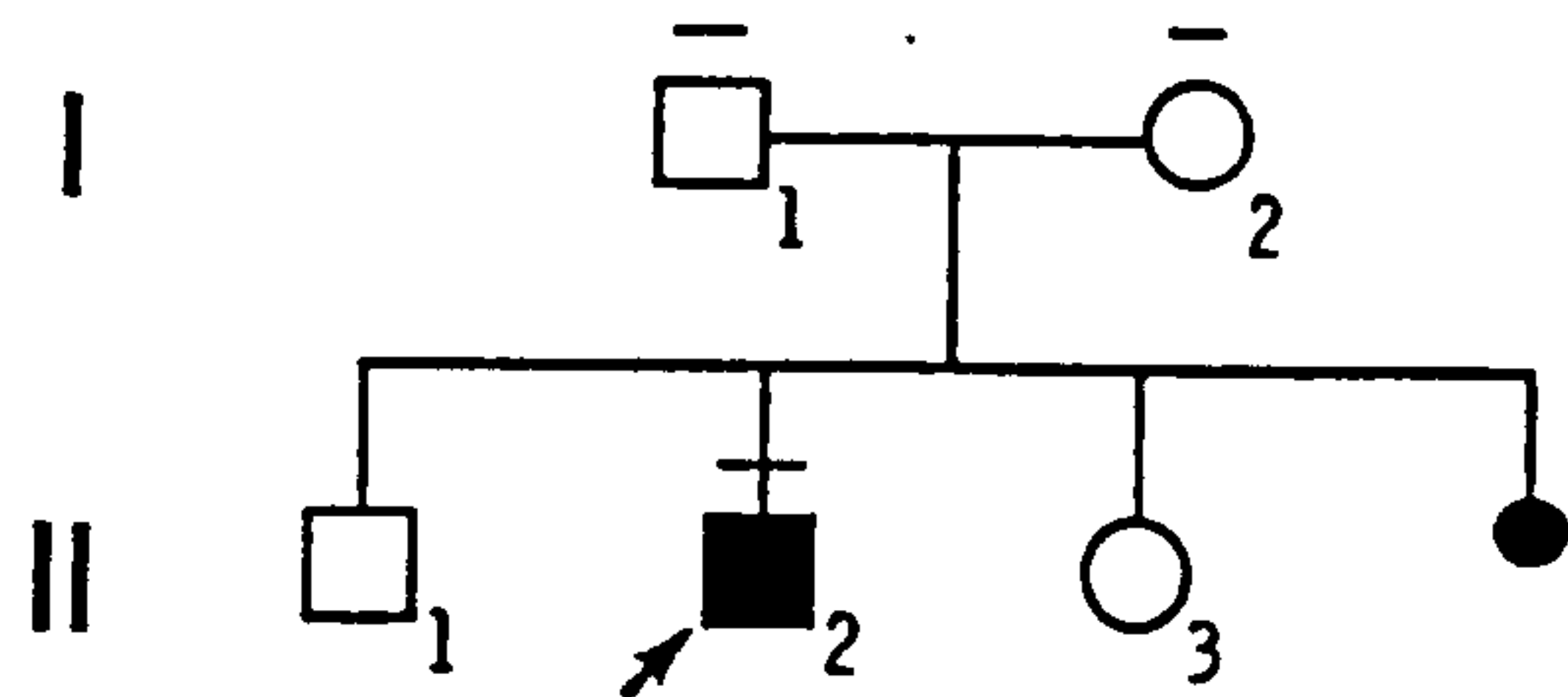
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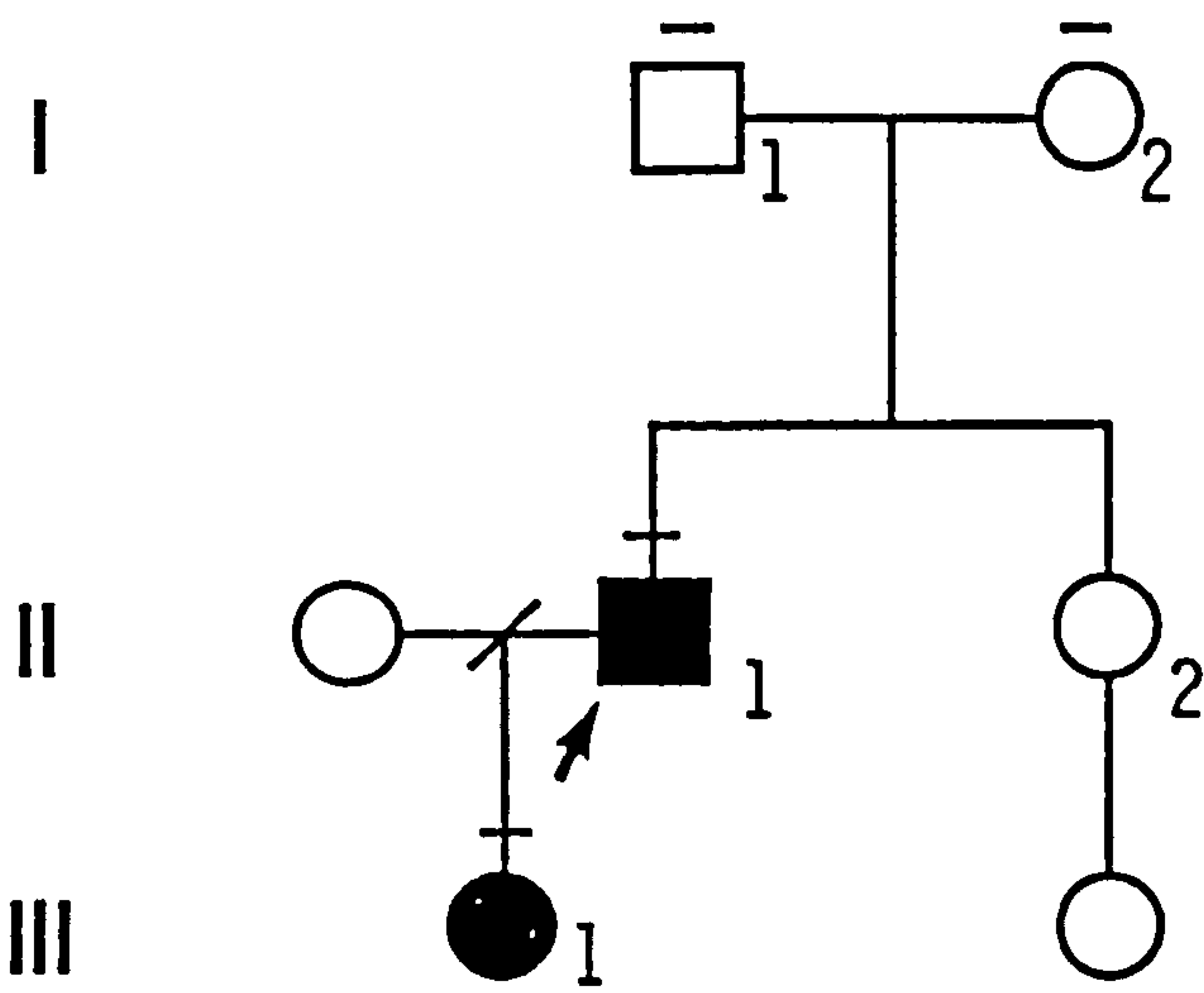
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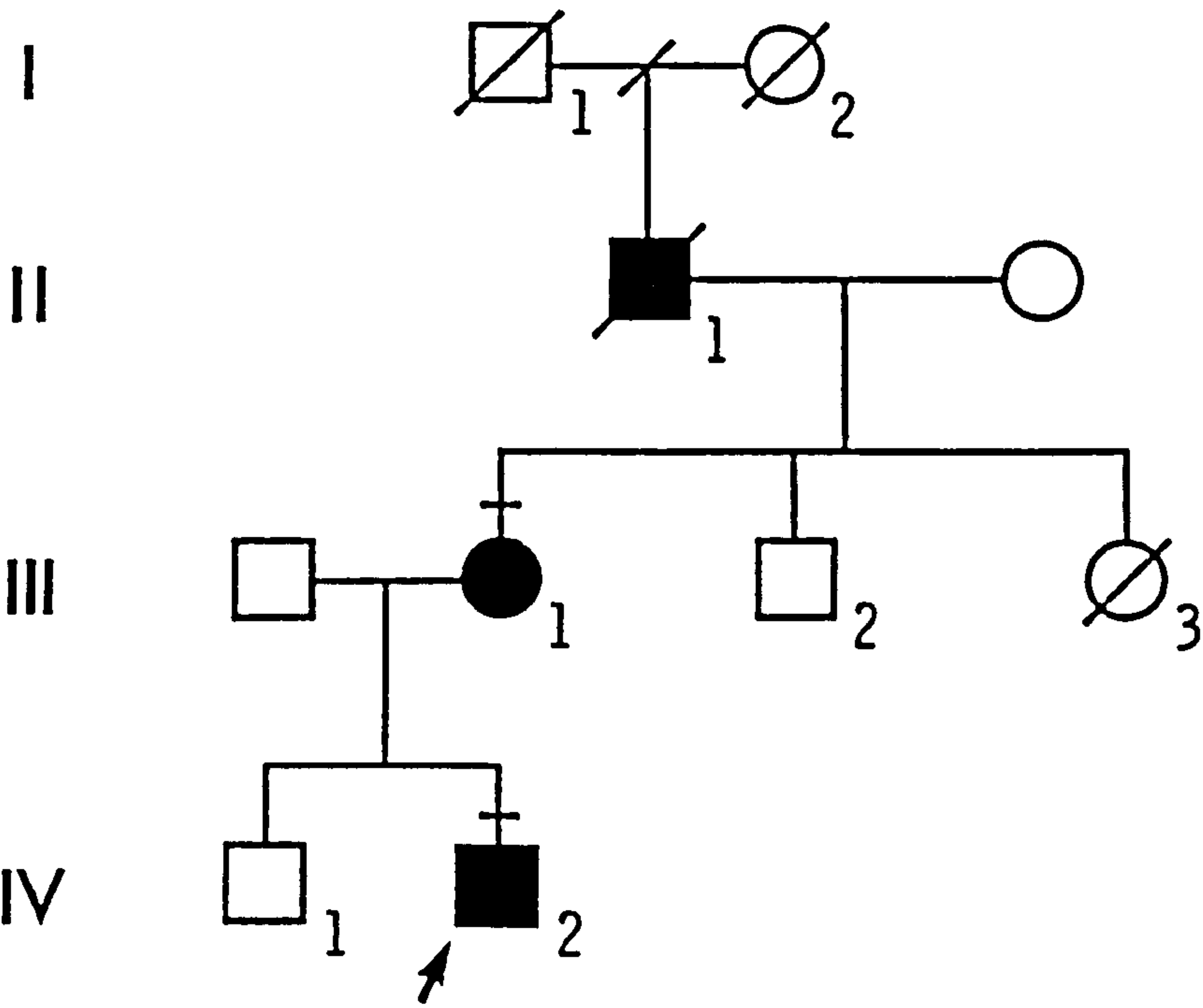
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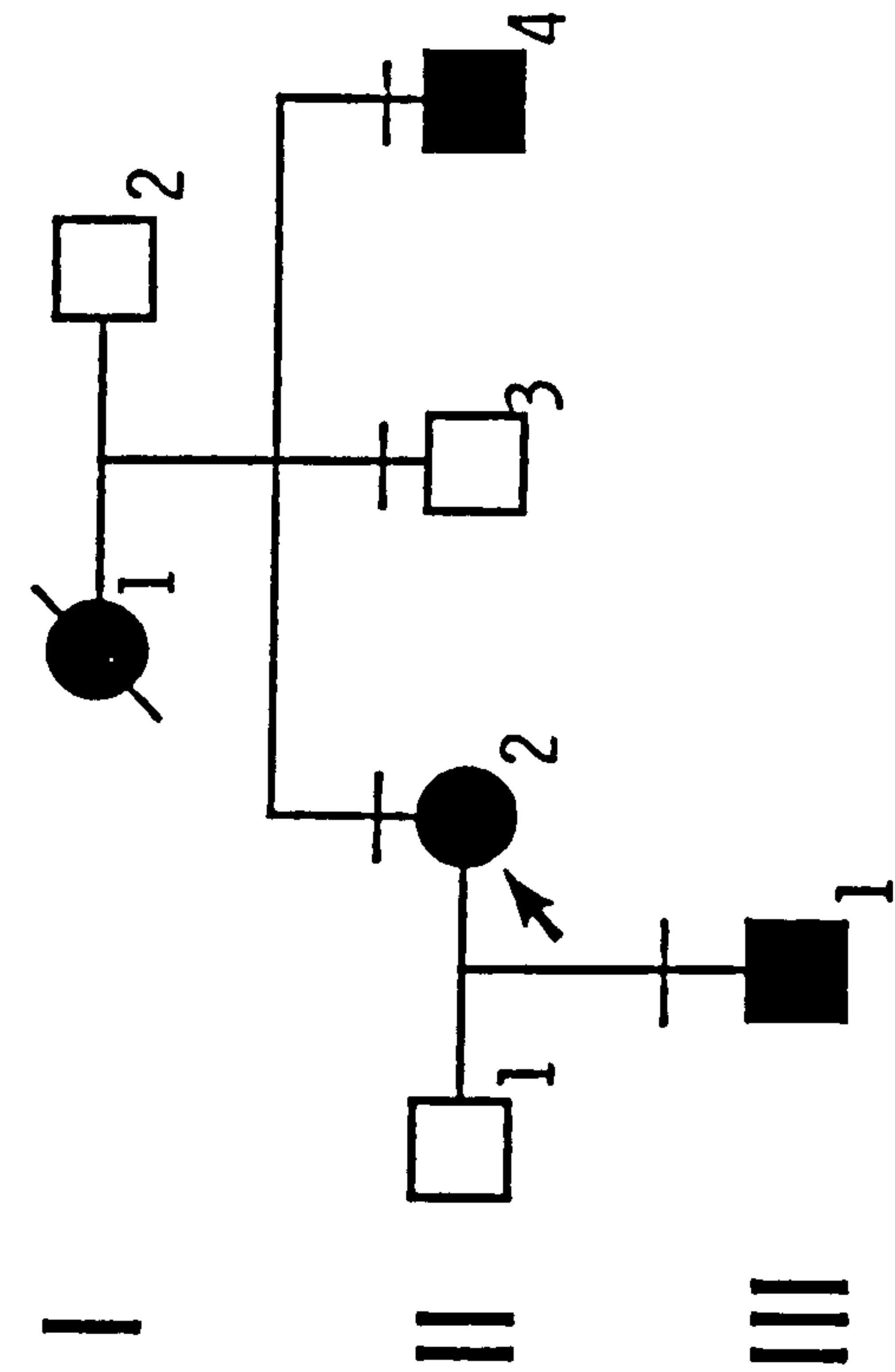
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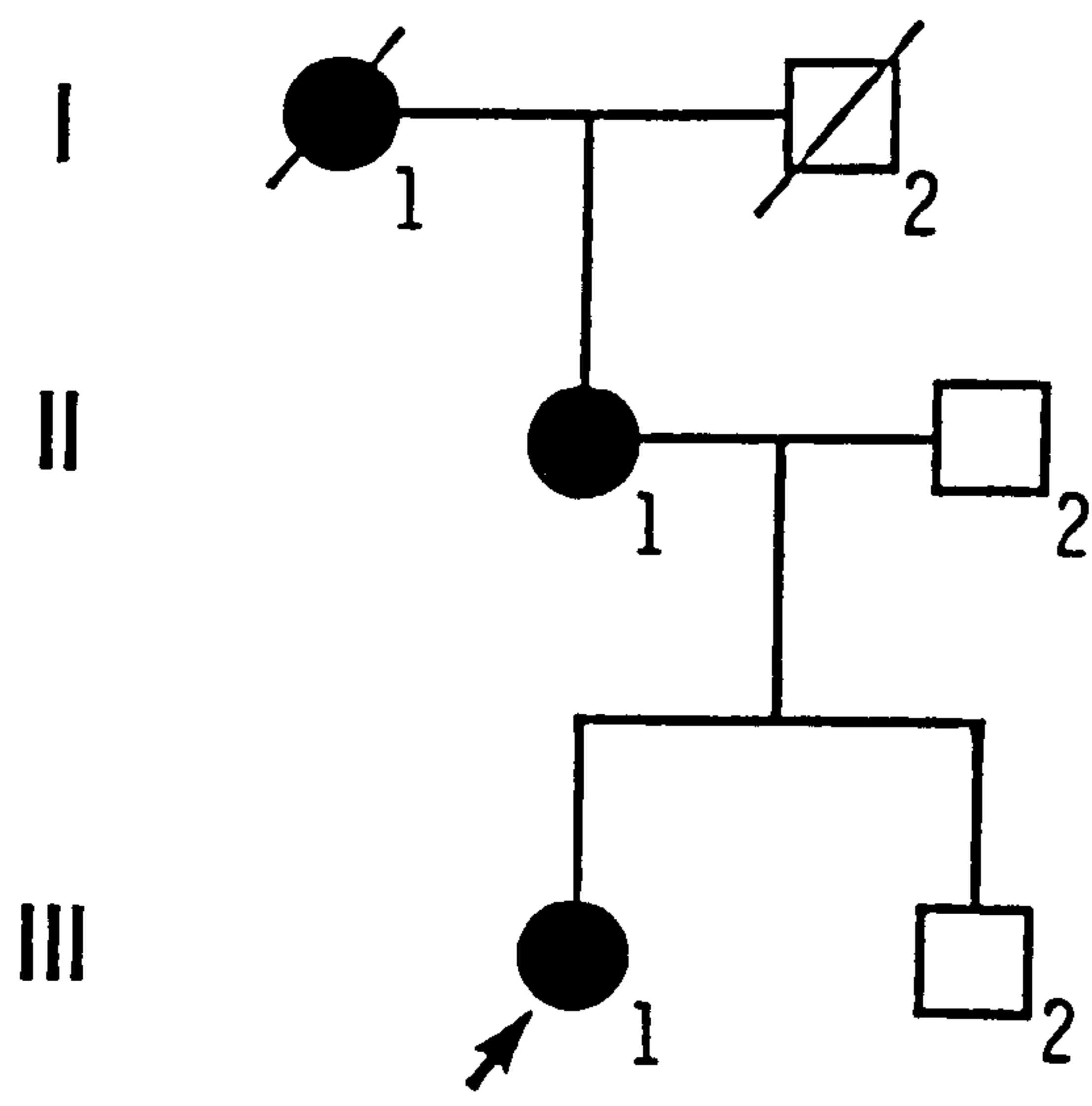
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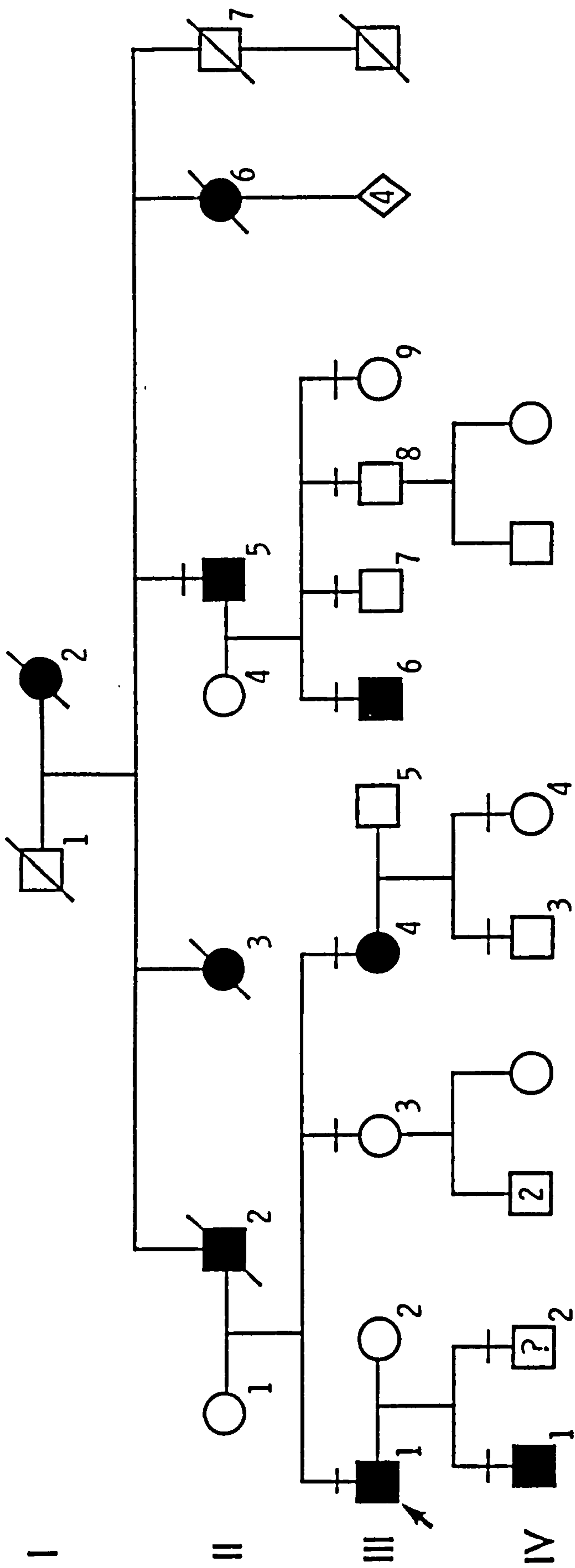
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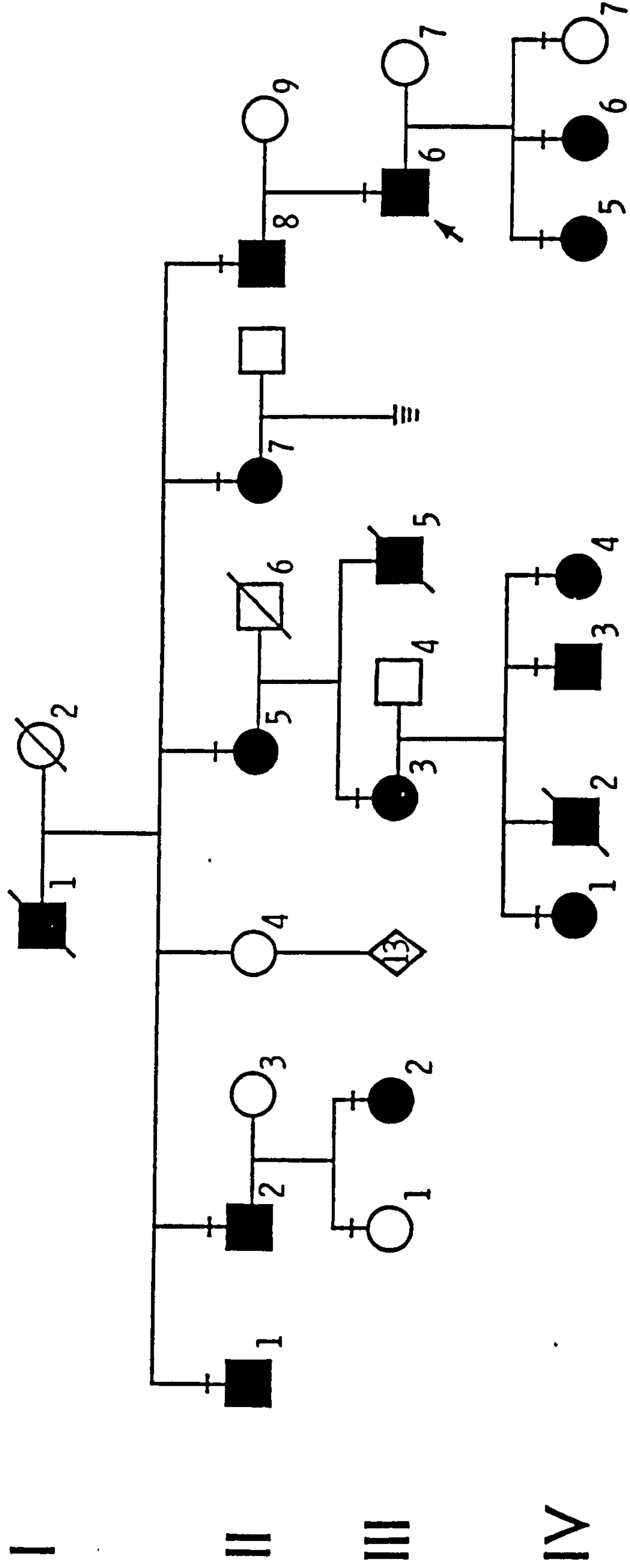
NF71



NF72



NF73



Section 2

In this section, the four index cases who had a negative family history and CAL spots as their only feature are described. Relevant details of other family members are supplied in the text, no pedigrees are provided.

DNF 1

The index case was referred by a consultant neurologist who noted the CAL spots on examination when she was referred for an unrelated problem.

Index case - D.o.b. 25.6.60, 24.5 years. On examination the only abnormality was 4 CAL spots >1.5 cm diameter; there was no freckling, neurofibromas or Lisch nodules and no relevant past medical history.

Family review - The parents of the index case (father aged 61, mother 62 years) and 3 siblings (sister 35 years, sister 27 years and twin brother 24.5 years) were all reported to be in good health with no NF-1 features. The index case did not want them to be examined.

Conclusion - The CAL spots were considered to be a variant of normal.

DNF 2

Index case referred to study by his general practitioner.

Index case - D.o.b. not documented, aged 30 years at time of assessment. On examination, 2 CAL spots >1.5 cm in diameter, no other features.

Family review - Negative.

Conclusion - 2 CAL spots as variant of normal.

DNF 3

The index case was ascertained through the records of the Prince of Wales Orthopaedic Hospital and visited because of the mention of 3 CAL spots at the time of admission for scoliosis surgery.

Index case - D.o.b. 14.9.61, 24 years. The index case had surgery at the age of 14 years for idiopathic thoracic scoliosis, no underlying bony abnormality. She had had no other medical problems. On examination she had 2 CAL spots >1.5 cm in diameter and no other features.

Family review - Parents (aged 47 and 46 years), 1 brother (aged 22 years) reported normal.

Conclusion - CAL spots as variant of normal with coincidental scoliosis.

DNF 4

The index case was referred to the study by her paediatrician, the diagnosis of NF-1 having initially been queried by her GP.

Index case - D.o.b. 16.2.85, reviewed at 0.8 years. Normal developmental milestones; only problem parents had noted was slight ptosis of the left eyelid, this had been present from birth and was apparently non-progressive.

On examination length and weight on 50th, head circumference on 97th centile for age. 4 CAL spots >1.5 cm in diameter, 6 CAL spots > 0.5 cm diameter; no other features, slit lamp examination not possible. The ptosis of the eyelid was confirmed but there was no obvious subcutaneous swelling which might have suggested a plexiform neurofibroma.

Family review - Father - D.o.b. 18.3.46. Examination of skin and iris normal.

Mother - D.o.b. 15.9.49. One CAL spot >1.5 cm diameter, no other features.

Brother - D.o.b. 14.1.83. Reported normal.

Conclusion - Probable new mutation for NF-1 but diagnosis cannot be made until other disease features develop.

Section 3

In this section the four index cases classified as having other forms of neurofibromatosis are described. In the 2 cases where there is a possible (ONF 1) or definite (ONF 4) family history, pedigrees are supplied.

ONF 1

The index case was referred to the case by her consultant neurologist.

Index case (II-1) - D.o.b.: 15.9.25, 64.5 years. II-1 developed progressive bilateral deafness with tinnitus from the age of 46 years. From the age of 60 years she developed episodic vertigo and increasing ataxia. Her deafness had been assessed by an ENT surgeon originally who diagnosed sensorineural deafness of unknown aetiology. In 1984 increasing ataxia led to neurological referral. On examination she was profoundly deaf, had bilateral horizontal nystagmus, an absent left and decreased right corneal reflex and a slight right lower motor neurone facial palsy. She was ataxic with increased tone and brisk reflexes in the legs. Cranial CT showed large bilateral acoustic neuromas but no other abnormality.

She underwent 2 operations for removal of the acoustic neuromas but was left with no hearing, bilateral facial palsies and swallowing difficulties. Her ataxia showed no improvement post-operatively. On examination for the study, her only cutaneous features were 2 CAL spots, LN were not present.

Family review -

I-2 - Born 1987, died aged 83 years in 1980. Increasing deafness with tinnitus from the age of 40 years. During the last year of life she had been bedridden because to ataxia presumed due to "old age". Cause of death unknown. No significant preceding family history, her husband had had no hearing problems.

Generation II - Because of the possibility that I-1 had NF-2 her siblings were contacted. II-4 had died of pulmonary TB at the age of 30 years with no preceding relevant symptomatology. Her remaining

siblings all reported no hearing problems and all had normal audiological assessments at the time of the study.

Generation III - The children of II-1 were all asymptomatic but underwent examination and screening for acoustic neuromas with audiometry, brain stem auditory evoked responses (BAER) and CT scan.

III-1 - D.o.b. 27.3.47, 37 years. Normal cutaneous examination and screening investigations.

III-2 - D.o.b. 9.1.51, 33.2 years. 2 CAL spots on examination. Decreased right sided caloric response but BAER normal; cranial CT with contrast showed possible small right sided acoustic neuroma, not confirmed on more detailed CT using air contrast.

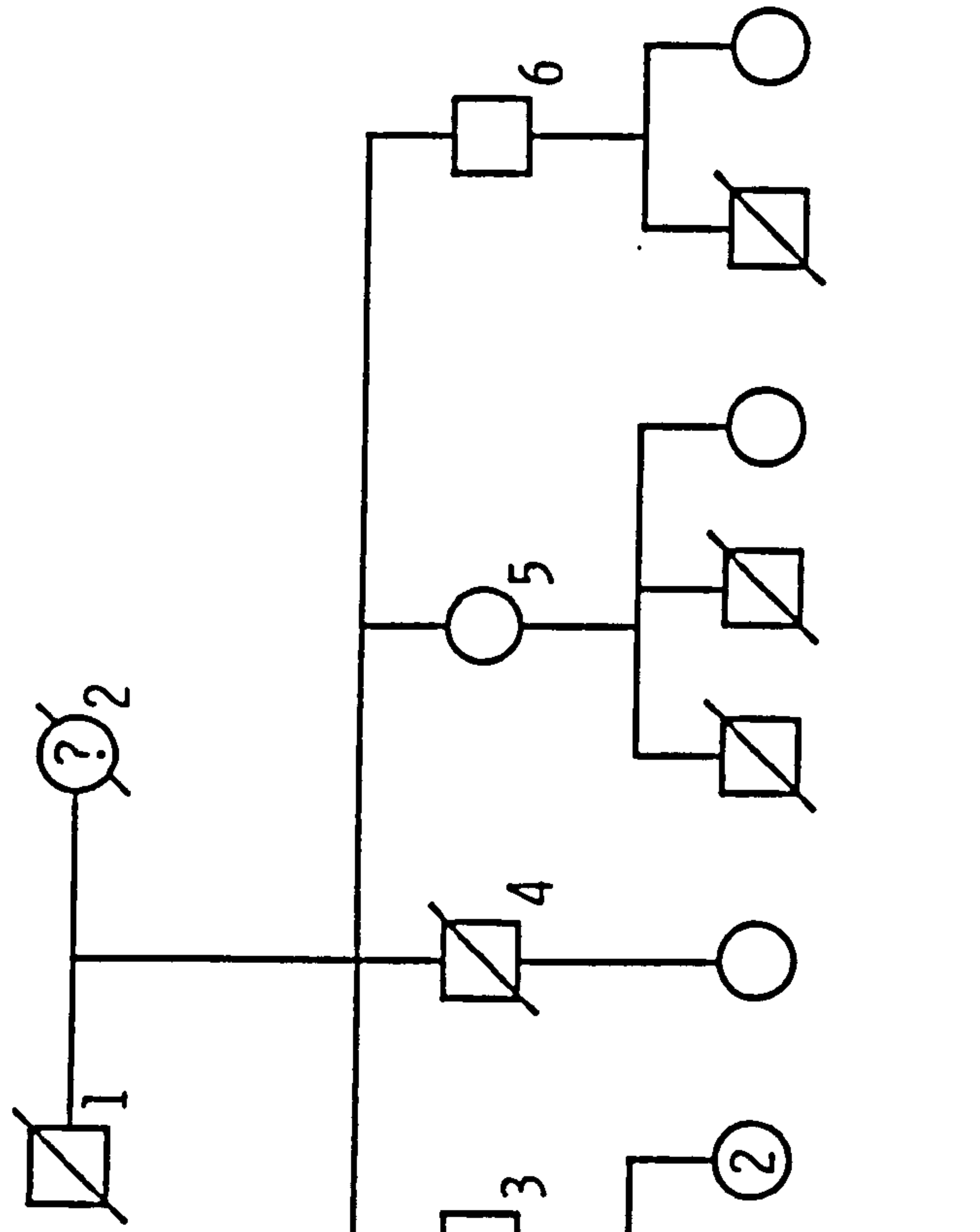
III-3 - D.o.b. 9.4.61, 24 years. 1 CAL spot on examination. Normal audiometry and BAER testing; cranial CT with contrast showed possible small left intra-canalicular acoustic neuroma.

Other members of generation III - The other members of generation III were reported to be asymptomatic. The causes of death in the 4 deceased individuals were not indicative of being related to NF-2.

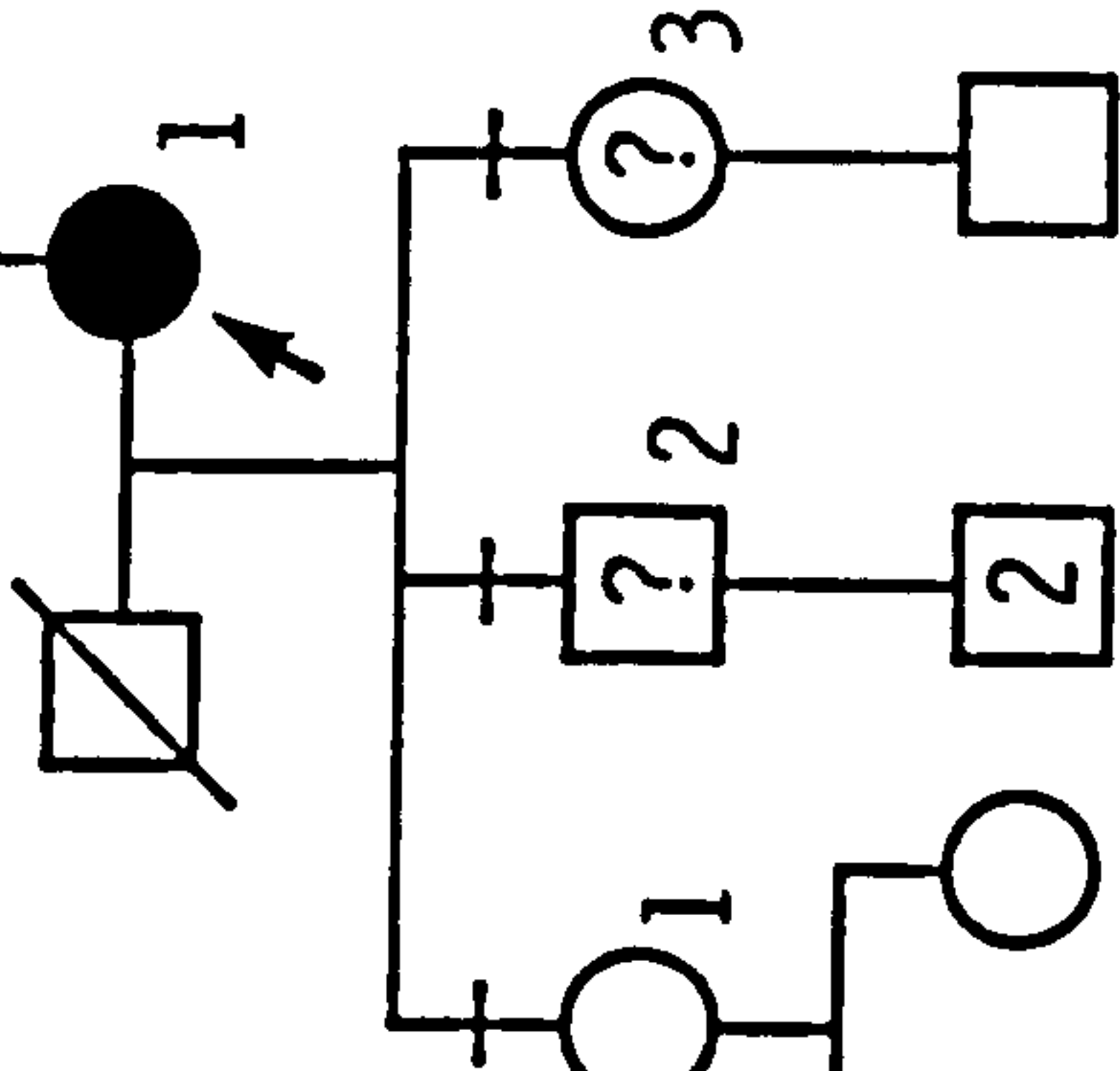
Conclusion - II-1 has NF-2; the problems experienced in diagnosis and management are not unusual (Huson & Thrush 1986). Following the initial assessment of her children, arrangements were made for them to have annual follow-up.

ONF1

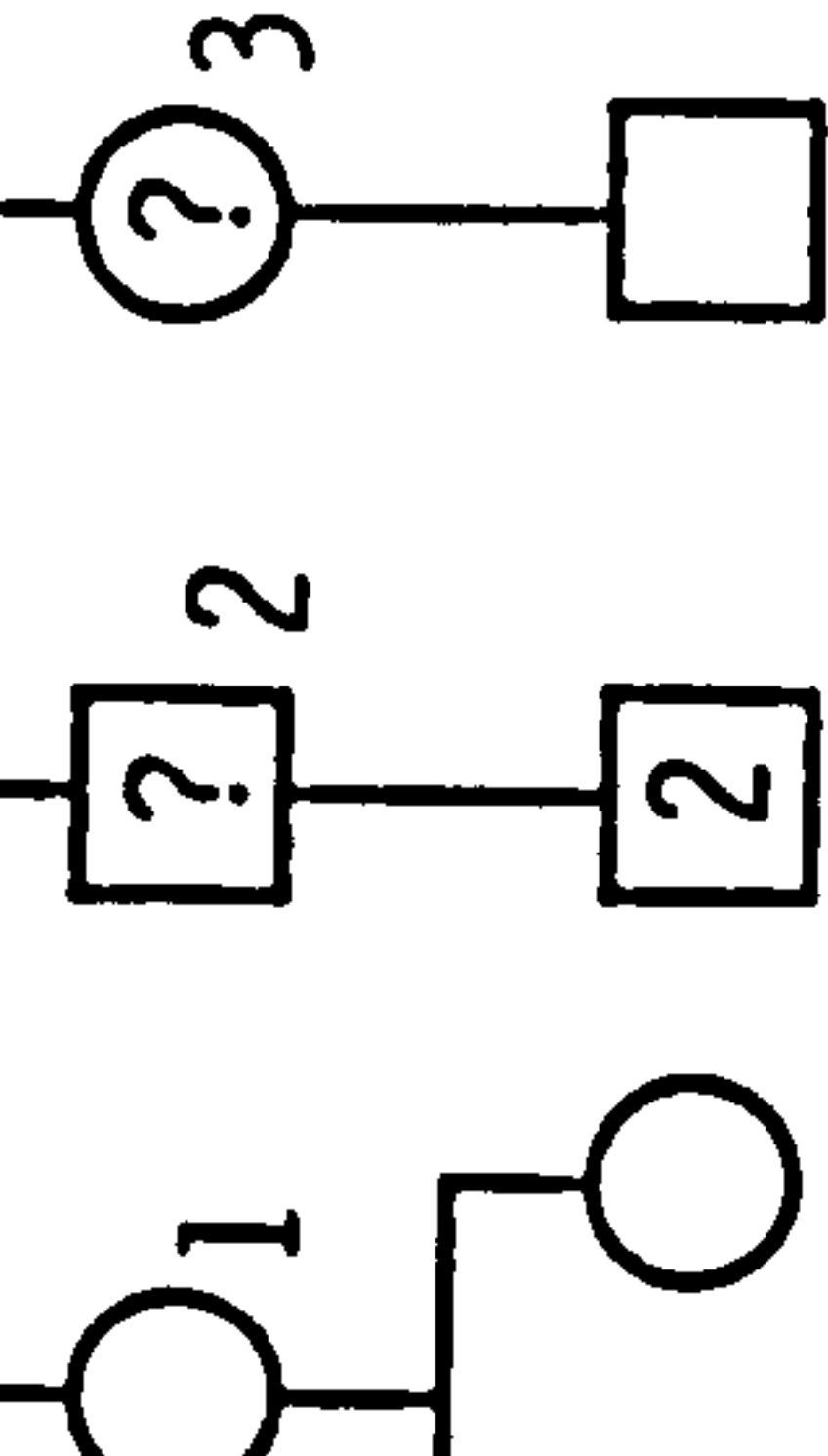
I



II



III



IV



ONF 2

The index case was referred to the study by his GP.

Index case - D.o.b. 19.11.42, 42.8 years. The only health problems the index case had had were a series of subcutaneous lumps which developed from his mid-30s onwards, some of which had given rise to severe paraesthesiae if knocked. He had had 6 lesions removed - 4 from his forearms, one from the back of his neck and one from his right flank. Histology reports were of neurofibromas for 2, lipomas for 3 and non-specific histology for the 6th lesion.

On examination he had no CAL spots or LN. He had 3 very firm, discrete subcutaneous nodules, 2 of which (on his left forearm and groin) were acutely painful when pressed. Clinically they were more like neurofibromas than lipomas, but further biopsy was not felt to be justified. No other abnormalities were found on clinical examination.

Family review - The index case reported that neither his parents nor 5 siblings had similar problems.

Conclusion - One neurofibroma can develop as an isolated event in otherwise healthy individuals. The index case in ONF-4 however has had 2 definite neurofibromas removed and has a further 3 on examination, which is unusual. He had no features to suggest he has these lesions as stigmata of NF-2. Riccardi (1982) has described a form of neurofibromatosis where the individuals develop cutaneous +/- spinal neurofibromas in adult life. It may be that the index case in ONF-2 falls into this category.

ONF-3

The index case was referred to the study by her GP.

Index case - D.o.b. 29.5.35, 50 years. This lady said she had had recurrent pain in her lower back since the age of 7 years. This had become an increasing problem since the birth of her first child in 1961 when the pain had begun to radiate into her left buttock and

leg. In 1969 (aged 34 years) the pain became more localised to the left buttock, more constant in nature, and was worse just before a period. She noted two lumps on the upper medial aspect of her left leg and was referred to an orthopaedic surgeon. The 2 lesions were removed and found to be neurofibromas. From that time the patient had had a number of further operations for the removal of many similar lesions from the left buttock and perineum. The lesions were usually multiple, and aggregating in 'strings' along nerves, with the histological appearance of a plexiform neurofibroma. Despite the operations there was little relief of pain; a myelogram undertaken as further investigation in 1976 was normal.

At the time of assessment for the study the patient still experienced severe left buttock pain radiating down the left leg; there were no palpable masses. Neurological examination was normal apart from the subjective alteration of appreciation of pin-prick and touch in the left leg. A pelvic CT undertaken to further define the lesion showed a soft tissue mass deep to the gluteal muscles in the fat of the lateral aspect of the ischio-rectal fossa with extension into the subcutaneous fat.

On cutaneous examination there were no features of NF-1.

Family review - Index case reported no similar problems in preceding generations or her siblings. Her children (girls aged 24, 22, 20 years, boy aged 16 years) were all personally examined and had no NF-1 features.

Conclusion - The index case has an isolated plexiform neurofibroma apparently involving the left-sided coccygeal plexus and possible upper branches of the sciatic nerve on the left side. Plexiform neurofibromas are unusual occurring in isolation, it could be possible that they are similar in aetiology to cases of segmental neurofibromatosis; it has been suggested that these represent a somatic mutation of the NF-1 gene (Carey 1986).

ONF 4

The index case (II-2) was referred to the study by his GP.

II-2 - D.o.b. 10.10.25, 59.6 years.

II-2 developed low backache and bilateral sciatica in his mid-teens and was initially thought to have 'arthritis'. At the age of 30 years he was referred for neurosurgical assessment because of numbness in the buttock and because plain X-rays had shown evidence of erosion at the pedicles suggesting an expanding spinal cord lesion. Examination showed mild diffuse weakness of the lower limbs with decreased right and absent left ankle jerk, analgesia over the buttocks and S1 bilaterally.

Myelography showed a large lesion in the cauda equina, another in the upper lumbar/lower thoracic region and possibly an upper thoracic lesion. He initially had a massive lesion removed from the cauda equina but as the nerve roots tracked through and around the tumour, some were damaged. He was left with partial paralysis of the left leg and dribbling incontinence. Tumour histology was of a schwannoma.

At a second operation a further tumour was removed from the lower thoracic region. He had had two further explorations of the lumbar region since that time (1963 and 1967) both precipitated by increasing pain and weakness. On the first occasion no abnormality was found apart from arachnoiditis and on the second a further tumour was removed. Following the second operation he had been relatively static with regard to his degree of disability. On examination at the time of the study, he had no cutaneous features of NF-1. Neurological examination showed no abnormality in the cranial nerves or upper limbs. He was incontinent of urine with occasional incontinence of faeces, had diffuse weakness of his lower limbs, more marked distally with absent lower limb reflexes. He reported decreased sensation from the buttocks downwards but this was not formally examined as he was seen at home.

Family review

I-1 and I-2 - His parents had both died of ischaemic heart disease with no neurological symptoms prior to death.

II-1 and II-3 - D.o.b. 25.6.23 and 23.9.27 respectively. Reported normal.

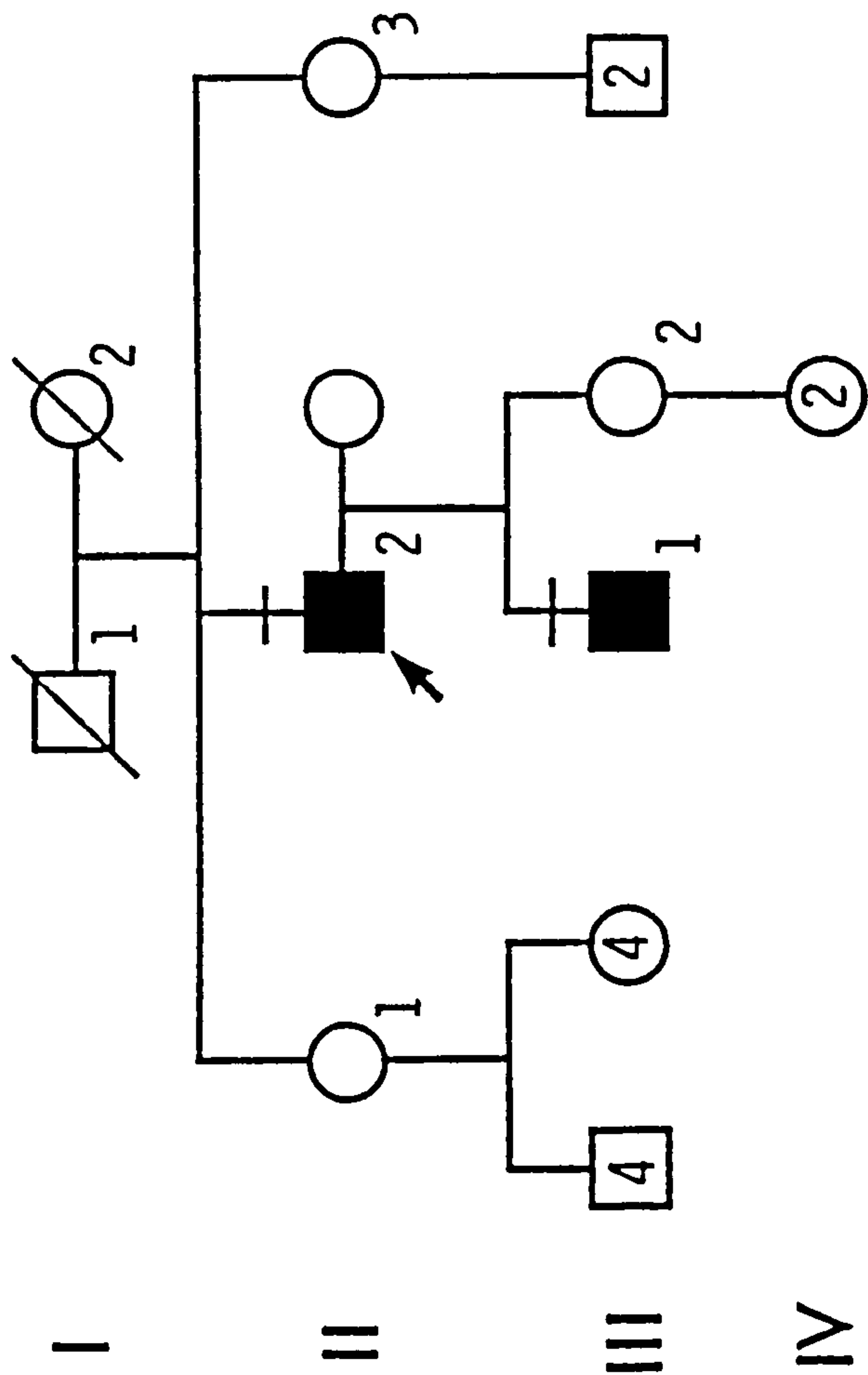
III-1 - D.o.b. 28.9.54, 30.8 years. III-1 had developed recurrent episodes of pain in the neck from 18 years of age, in 1978 he developed paraesthesia in the left hand and weakness of the left leg. He was referred to a neurosurgeon and myelography showed an extradural spinal tumour in the cervical spine. This was successfully removed with complete resolution of his symptoms. Histology was of a schwannoma.

He had had no further problems at the time of assessment. Examination showed no cutaneous or neurological abnormalities.

III-2 - D.o.b. 27.3.56. Reported normal.

Conclusion - It was felt that II-2 and his son have a dominant gene predisposing to multiple spinal schwannomas. Although this is a common feature in families with NF-2, the latter diagnosis seems unlikely as neither of the cases have clinical evidence of acoustic neuromas, these are usually symptomatic by the mid-20s in patients with NF-2 (Kanter *et al* 1980). We therefore concluded that ONF-4 may represent a further variant form of neurofibromatosis.

ONF4



Section 4

In this section, the 16 index cases in whom diagnoses other than NF-1 were made are reported. Relevant details of other family members are supplied in the text and there are no accompanying pedigrees.

NNF-1

The index case was ascertained through hospital records.

Index case - D.o.b. 9.11.28, 57 years. This man had developed multiple painless subcutaneous swellings on his trunk from his 30s onwards. In 1983 had had been referred for hospital consultation when it was felt that the lesions were either neurofibromas or lipomas; biopsy was not undertaken as he was asymptomatic. On examination for the study, the patient's lesions were thought clinically to be lipomas, he had one CAL spot but no other abnormalities. Family history was negative.

Diagnosis - Multiple lipomas.

NNF-2 - The index case was referred by his GP.

Index case - D.o.b. 12.7.52, 33 years. This man had noted 3 painless subcutaneous lumps for approximately 6 years. On examination 3 lesions were on his left forearm, anterior chest and right hip; they were approximately 2 cm in diameter and were thought clinically to be typical of lipomas. There were no other cutaneous abnormalities and the family history was negative.

Diagnosis - Multiple lipomas.

NNF-3 - The index case was referred by her GP.

Index case - D.o.b. 18.4.48, 37.3 years.

This lady had recently developed 2 painless subcutaneous swellings, one on each forearm. Clinically they were small lipomas. No other abnormality on examination. Family history was negative.

Diagnosis - Lipomas.

NNF-4

The index case was referred by his GP.

Index case - D.o.b. 12.2.20, 65.5 years.

This man had developed multiple painless subcutaneous lesions on his forearms and trunk from his late 20s. Clinically these were lipomas; no abnormality on cutaneous examination. No significant family history.

Diagnosis - Multiple lipomas.

NNF-5

Referred to the study by another patient.

Index case - D.o.b. 3.8.33, 55.5 years.

This man had a family history of multiple lipomatosis, his mother and 2/3 siblings being affected. He had approximately 20 lesions on his forearm and trunk; he had had one removed in the past and histology showed a lipoma.

Diagnosis - Multiple lipomas.

NNF-6

This case was ascertained via hospital records.

Index case - D.o.b. 15.7.05, 79.8 years.

NF-1 had been diagnosed on clinical grounds when he had been

investigated for haemoptysis in 1976; chest X-ray had showed a well-defined right upper lobe lesion and several subcutaneous nodules had been noted on his forearms and chest. The chest lesion showed no change in 6 months and the patient refused an operation. He had remained well and on examination for the study he had 5 painless subcutaneous swellings with the clinical appearance of lipomas and no other cutaneous abnormality. The patient could not remember his parents having similar lesions, although he reported that one of his 8 siblings did.

Diagnosis - Multiple lipomas with ?solitary thoracic neurofibroma or lipoma.

NNF-7

Referred to the study by his GP.

Index case - D.o.b. 15.11.44, 40.6 years.

This man had developed multiple subcutaneous lesions on his forearms and trunk from his early 30s. Clinically they were not painful on palpation, had a soft consistency and were 2-3 cm in diameter, i.e. they were more typical of lipomas than neurofibromas. The only other cutaneous abnormality was one CAL spot. Negative family history.

Diagnosis - Multiple lipomas.

NNF-8

Referred by GP.

Index case - D.o.b. 6.1.47, 38.5 years.

This man had first noticed a painless subcutaneous swelling to the right of his lumbar spine at the age of 26 years. He had subsequently developed 3 further lesions, none of which caused any problems. Clinically they were lipomas rather than neurofibromas and there were no other abnormalities on cutaneous examination.

His father and 1/3 brothers were reported to have similar lesions.

Diagnosis - Multiple lipomas.

NNF-9

This patient was ascertained via hospital records.

Index case - D.o.b. 207.35, 50.3 years.

This man had developed multiple painless subcutaneous lesions from the age of 26 years. On examination he had 10 lesions, on his forearms and trunk, which were thought to be lipomas; no other abnormality on cutaneous examination. Family history negative.

Diagnosis - Multiple lipomas.

NNF-10

Self-referral in response to newspaper article.

Index case - D.o.b. 19.5.28, 57.6 years.

This lady had 2 painless subcutaneous swellings, one on her knee and one on the left forearm; clinically they were lipomas. She had had them for a number of years and thought that in the past she had been told she had NF-1. No other findings on cutaneous examination. Family history negative.

Diagnosis - Lipomas.

NNF-11

Referred by GP.

Index case - D.o.b. 1.12.24, 60.8 years.

This man had started to develop multiple subcutaneous lesions from his early 30s. He had had one removed from his right forearm in 1979 and histology was of a lipoma. On examination he had

approximately 30 subcutaneous swellings which were clinically lipomas, and no other features.

The patient reported 1/6 siblings had similar lesions and that his son was also affected. Could not recall his parents having similar lesions.

Diagnosis - Multiple lipomas.

Summary of NNF 1-11 - The clinical distinction between lipomas and neurofibromas was found to be relatively straightforward. Although only a minority of cases had had biopsy proven lipomas, clinically their lesions and those of the other cases were identical. The main distinguishing features from cases with NF-1 were that the latter had cutaneous as well as subcutaneous lesions; subcutaneous neurofibromas were found along the trunks of peripheral nerves and were painful on palpation and that subcutaneous neurofibromas were firmer than lipomas and had more discrete margins.

NNF-12

Index case was a chronic schizophrenic who was referred by a psychiatrist. He had seen the possibility of NF-1 mentioned in her clinical notes on a hospital admission in the past and therefore referred her to the study.

Index case - D.o.b. 22.5.28, 57 years. On review this lady was found to have severe rheumatoid arthritis and her 'lumps' were rheumatoid nodules!

Diagnosis - Rheumatoid arthritis.

NNF-13 - Referred by GP.

Index case - D.o.b. not recorded. Age 50 years. This lady had multiple small moles on her neck and trunk but no other features. No other medical problems and negative family history.

Diagnosis - Multiple moles

NNF-14

This lady referred herself to the study in response to the newspaper article.

Index case - D.o.b. 5.6.39, 46.5 years.

This lady had had 'lumps' on her skin from childhood. They were painless, had increased in number throughout life, and frequently became infected. She had been told that she had NF-1 in the past.

On examination she had approximately 200 cutaneous lesions, 1-2 cm in diameter, which had a yellowish colouration and a firm consistency. These are shown in Figure A-1. There was no abnormal skin pigmentation. She clearly did not have NF-1 but as the diagnosis was unclear, she agreed to be seen by a dermatologist for biopsy. She was seen by Dr P Holt at the University Hospital of Wales; biopsy showed a cyst with the histological features of steatocystoma multiplex, a cystic disorder centred on sebaceous gland elements. This is an autosomal dominant disorder and one of the patient's children was similarly affected; the patient reported her sister, father and grandmother were also affected.

Diagnosis - Steatocystoma multiplex.

NNF-15

This patient was ascertained via hospital records.

Index case - D.o.b. 1.6.38, 47.4 years.

Neurofibromatosis had been diagnosed at the age of 15 years when she presented with a left thoracic scoliosis and a history of altered sensation over the left side of the body of a year's duration. She was referred to an orthopaedic surgeon and in turn to a neurologist. From the orthopaedic viewpoint she had a thoracic scoliosis, T4 - L1, with a curve of 46°, no underlying bony abnormality. The curve did not progress on follow-up. On neurological assessment, she was found

to have left-sided hyperalgesia with a left extensor plantar response. Multiple pigmented lesions were noted on her skin and a diagnosis of neurofibromatosis with possible intracranial involvement was made. The only abnormality on routine neurological investigation was slight increase in CSF protein, it was decided not to perform air encephalography but to observe her progress. The patient was then lost to follow-up.

When seen for the study, the patient still complained of altered left-sided sensation, but neither this nor her scoliosis had changed over the years. In the interim she had developed asthma and hypothyroidism and was on routine treatment for both. On examination she had multiple small, very dark, slightly raised lesions which were thought to be lentigines rather than CAL pigmentation (Figure A-1). This was confirmed on biopsy. She also had short stature, even accounting for the scoliosis, with a height of 142 cm (<3rd centile) and hypertelorism. Neurological examination showed subjective sensory changes on the left side with an extensor plantar. Cardiovascular examination was normal including ECG. The patient had no obvious hearing problems and neither audiology nor echocardiography was felt to be justified.

Putting the clinical features together, the diagnosis of LEOPARD syndrome was made; this would not account for the patient's neurological problems, the most likely explanation for which were a previous cortical infarction.

The patient reported that her father and one of her 4 siblings had similar skin pigmentation but no other abnormalities.

Diagnosis - LEOPARD syndrome

NNF 16

This patient was referred to the study by his consultant psychiatrist.

Index case - D.o.b. 17.10.67, 18.2 years. This patient was severely retarded, with limited speech and in long term hospital care. He was seen with his parents, neither of whom had any NF-1 features; there was no other significant family history.

The patient's main features were: severe retardation, slightly dysmorphic facies, short stature (height 150 cm, <3rd centile) and 3 large areas of CAL pigmentation with rather jagged edges. There were no other cutaneous features. This patient is shown in Figure A-1.

The diagnosis of NF-1 was excluded on clinical grounds but no other diagnosis was made. Further investigation included chromosome analysis both of lymphocytes and an involved area of skin, both of which were normal.

Diagnosis - Mental retardation with atypical CAL pigmentation and short stature - diagnosis unknown.

(A)



(B)



(C)

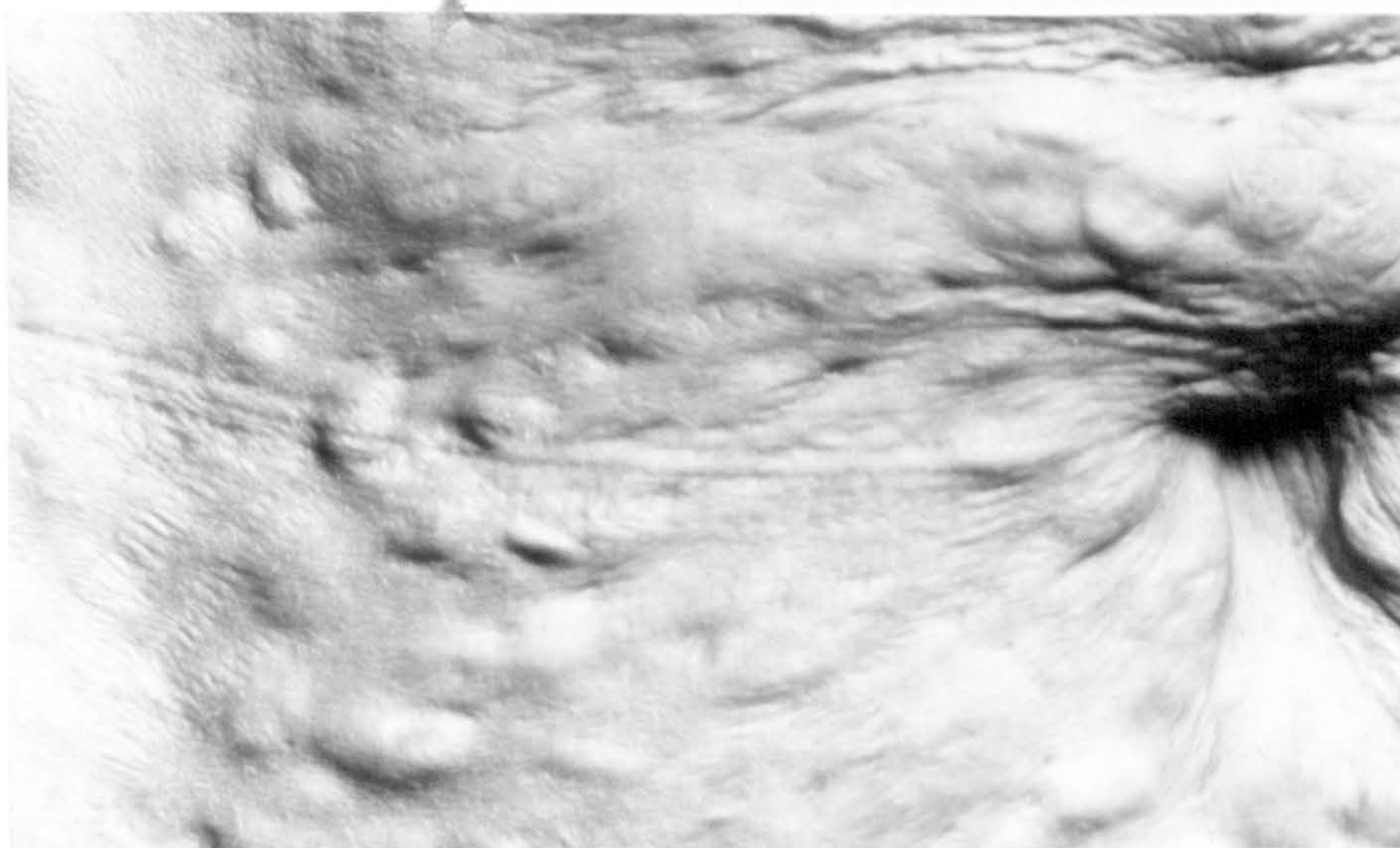


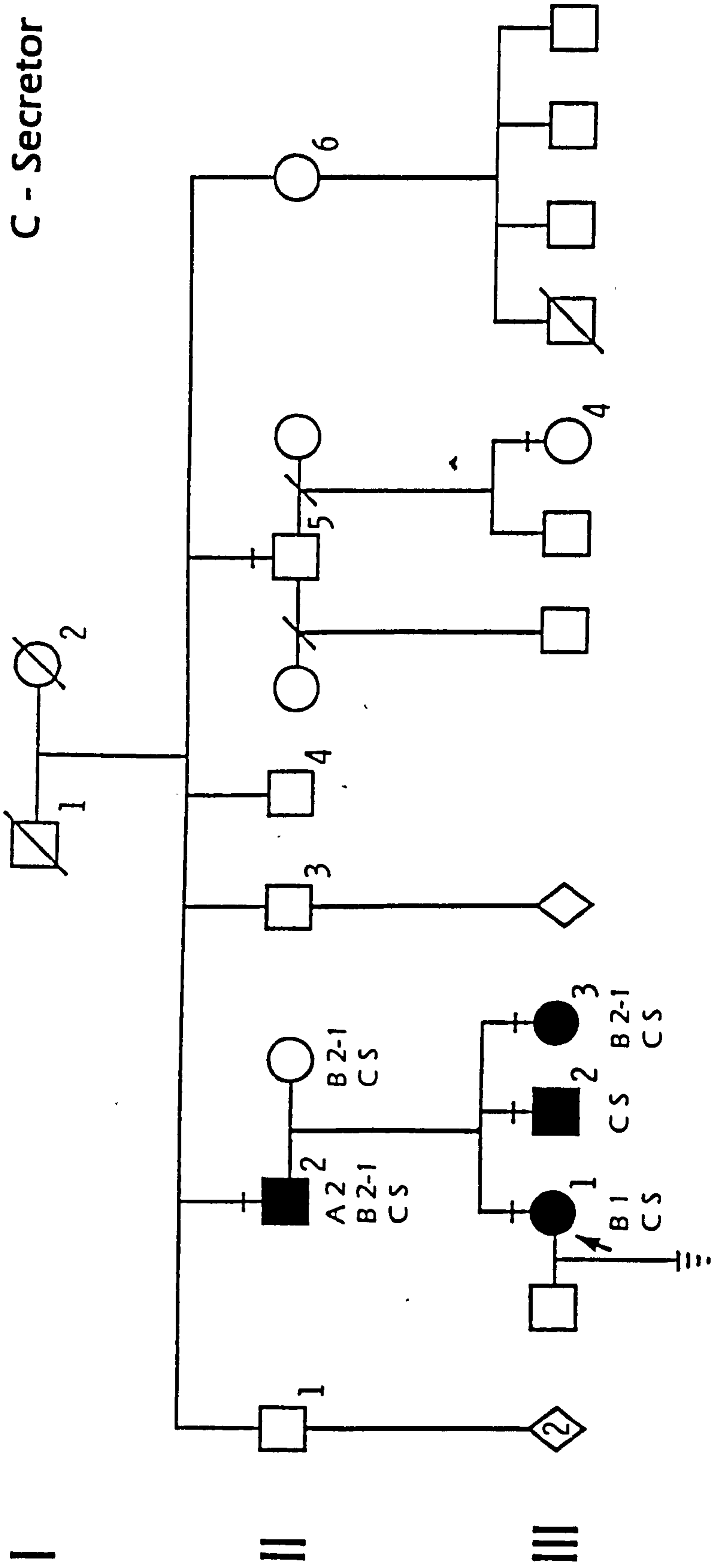
FIGURE A-1. A. LEOPARD syndrome (case NNF 15)
B. Atypical areas of CAL pigmentation in case NNF 16
in whom no diagnosis was reached.
C. Steatocystoma multiplex (case NNF 14)

APPENDIX B

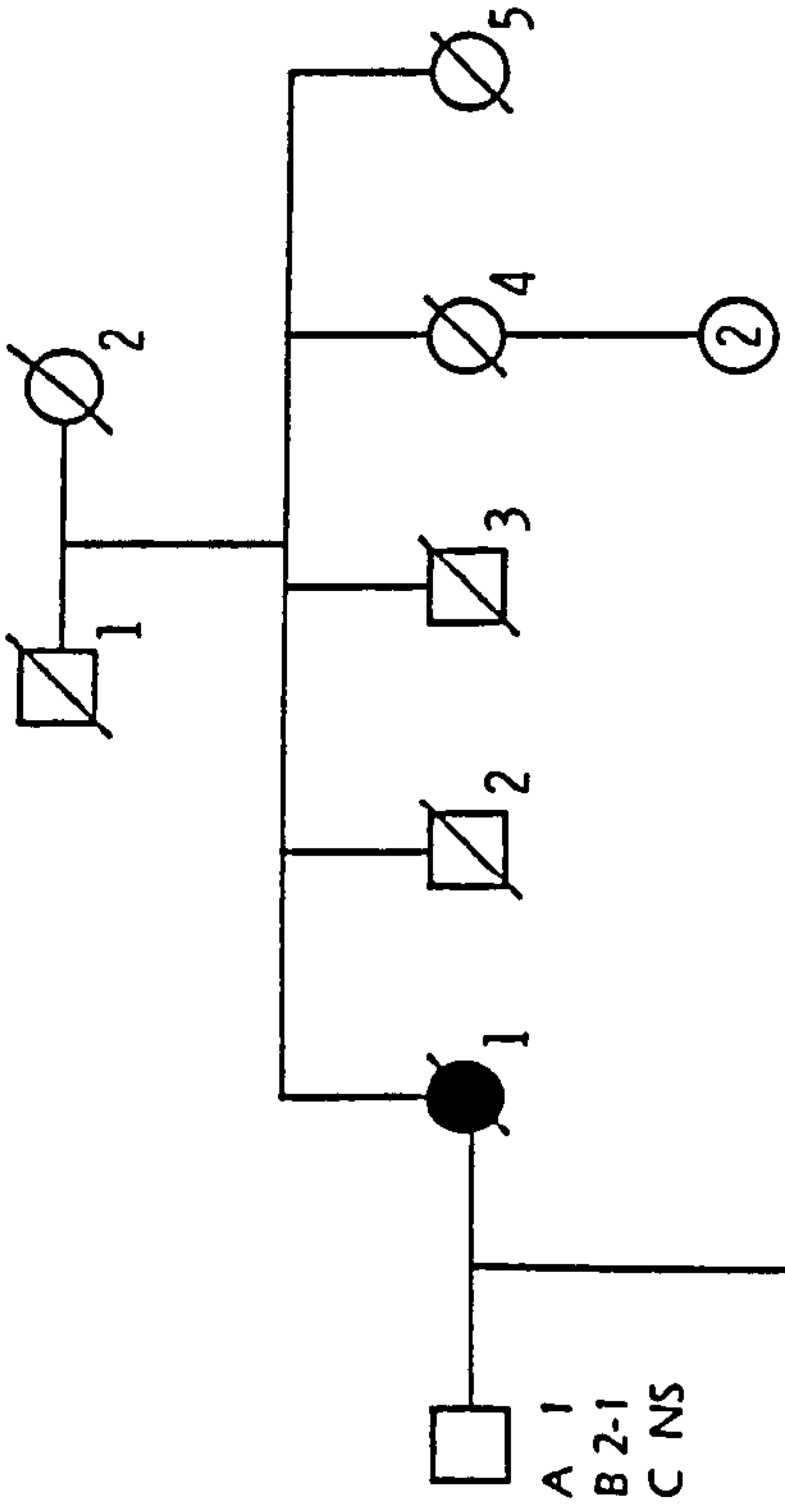
Genotypes for C3, APOC2 and Se polymorphisms in the 9 families used in the chromosome 19 linkage studies.

NF3

Key to markers
A - APOC2
B - C3
C - Secretor



I

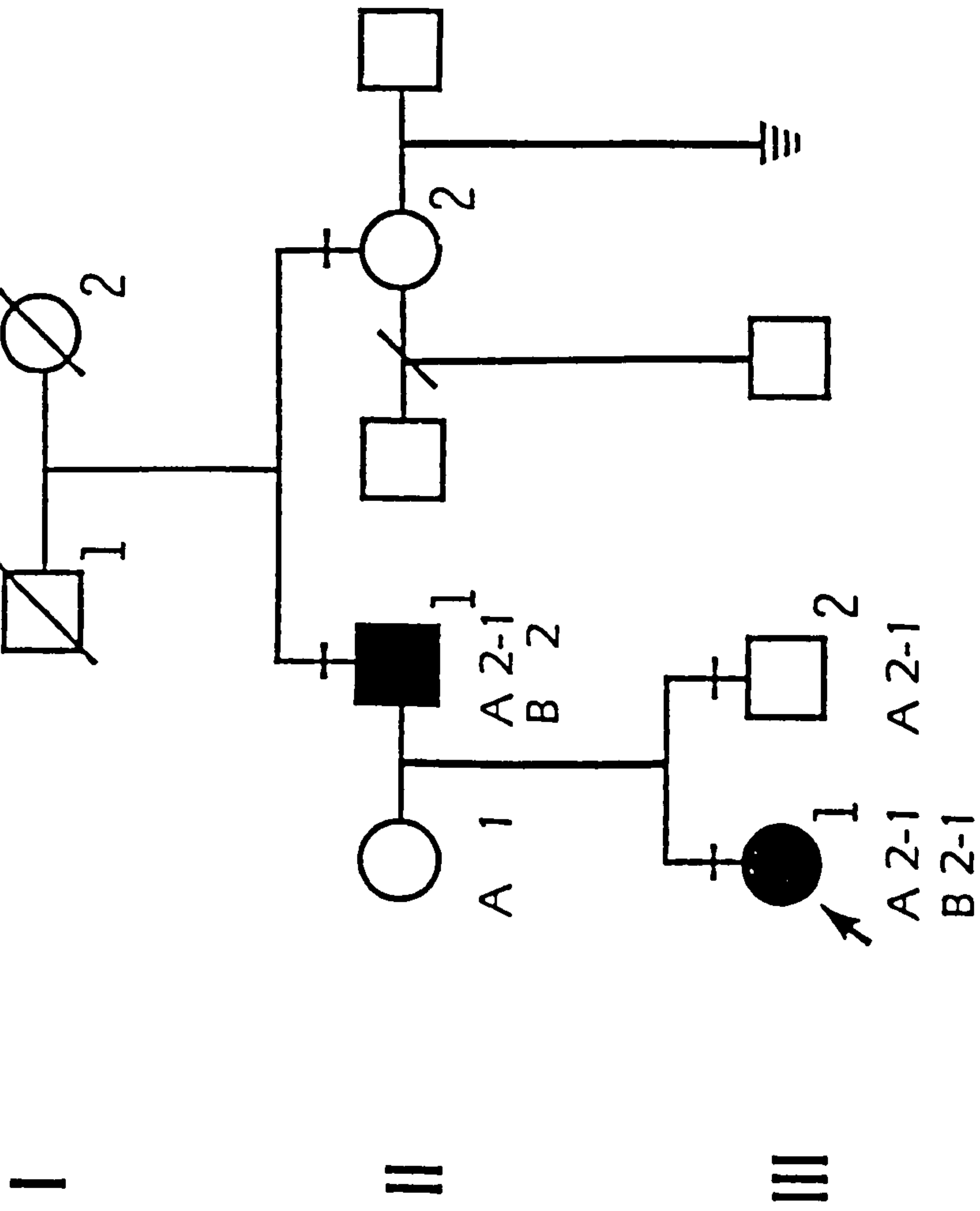


II

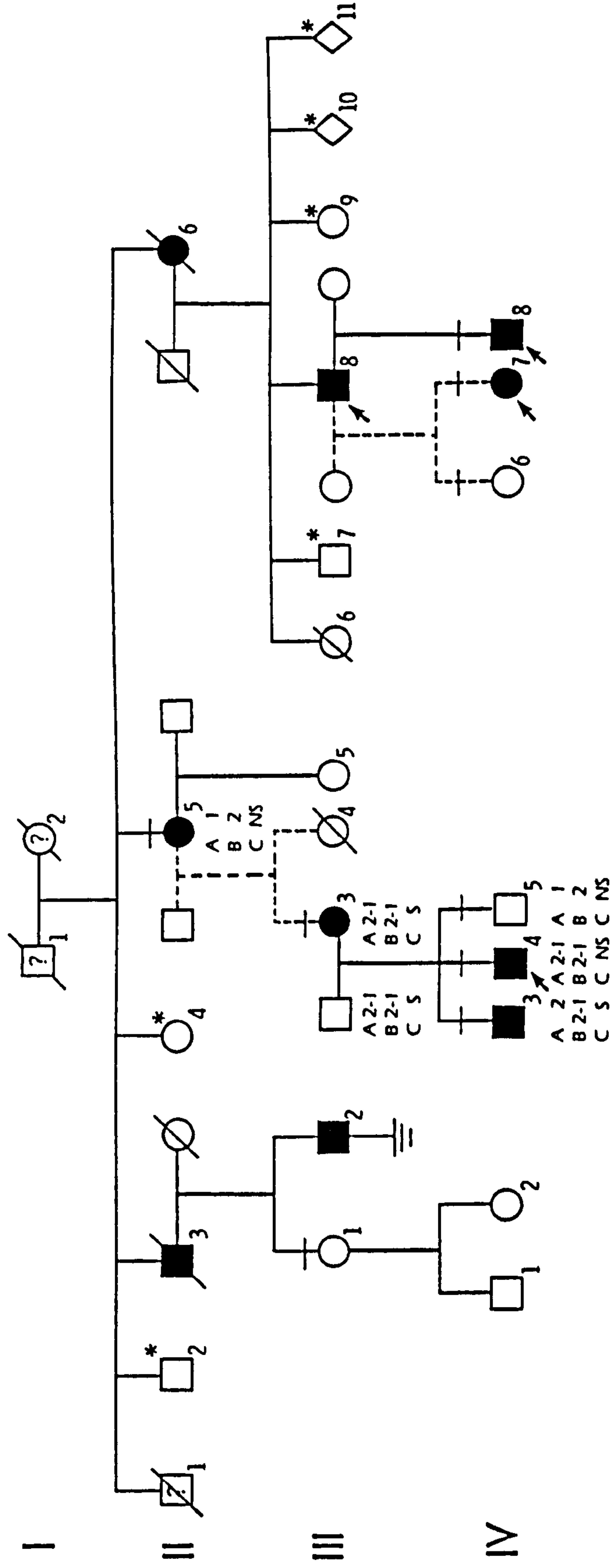
III

IV

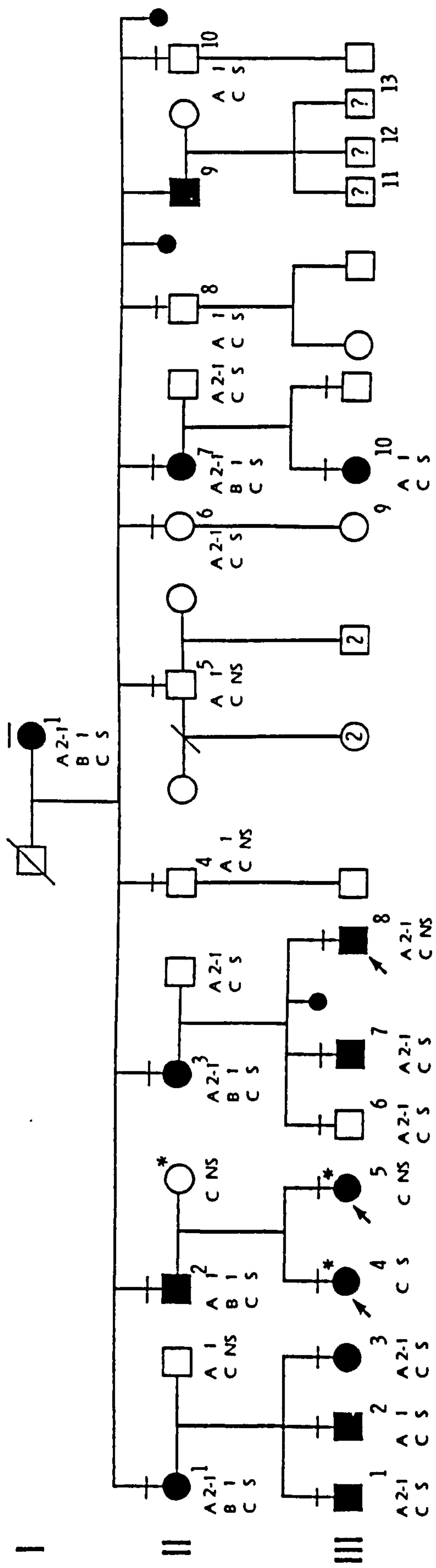
NF9



NF12

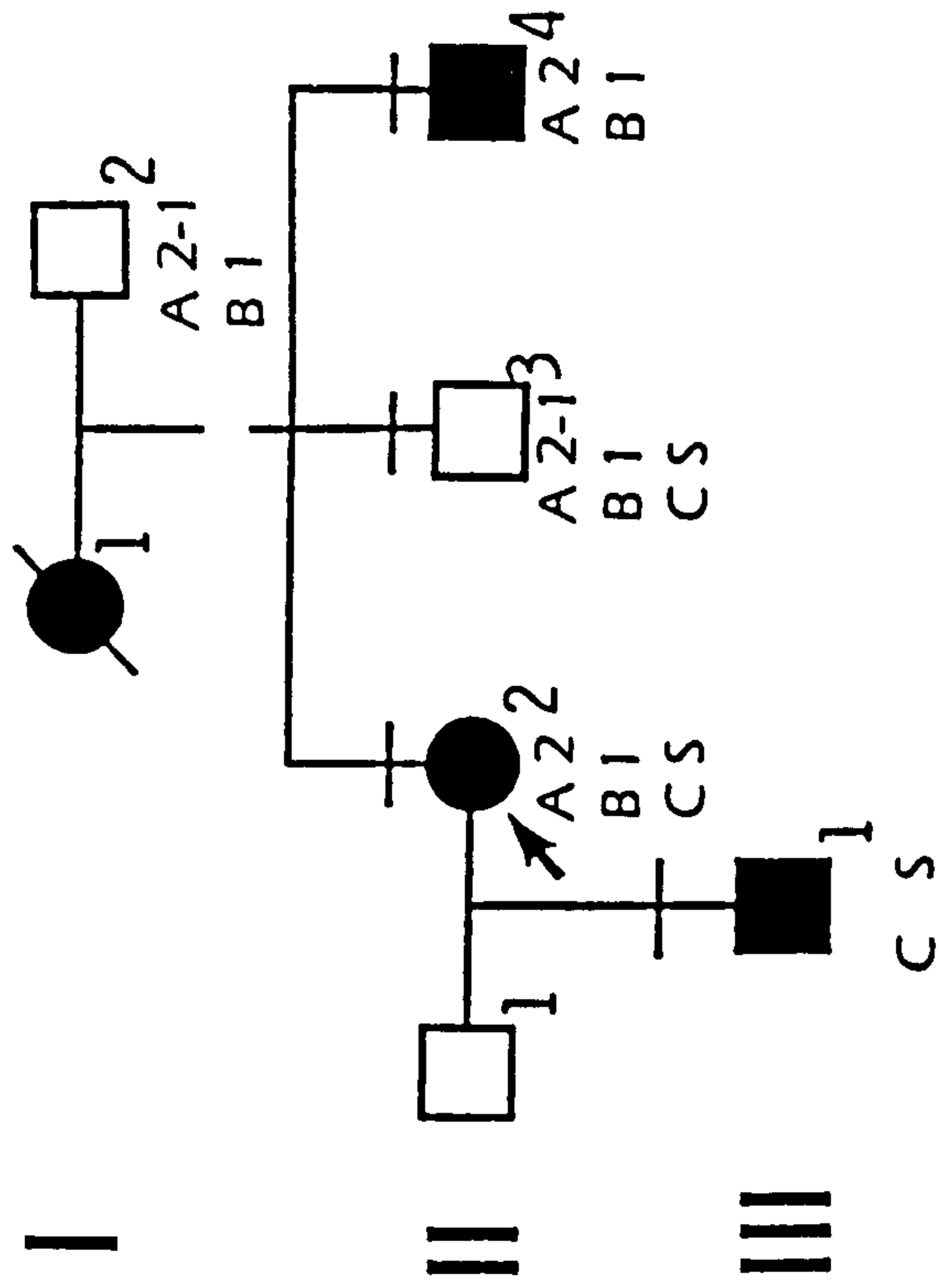


NF49

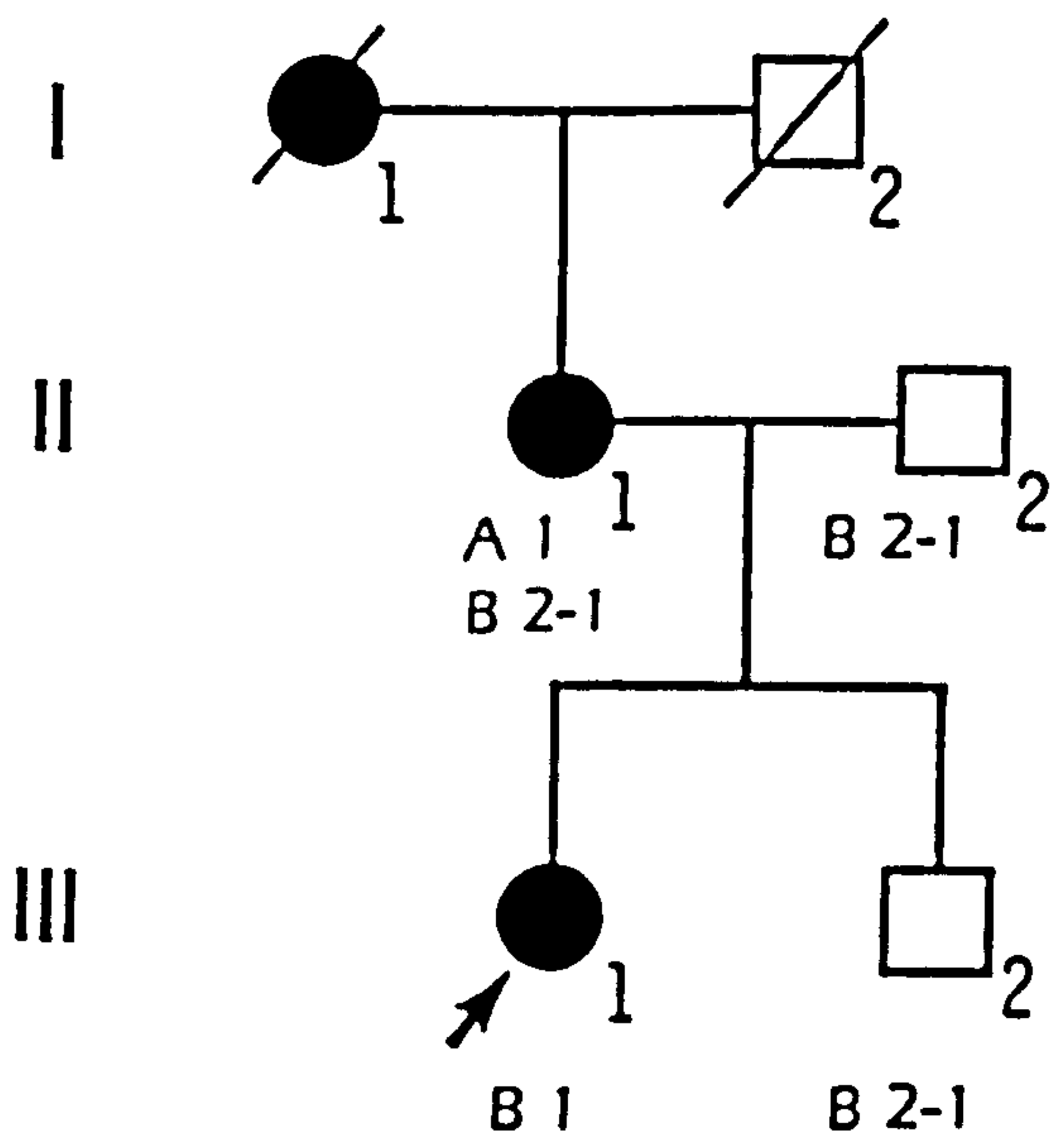


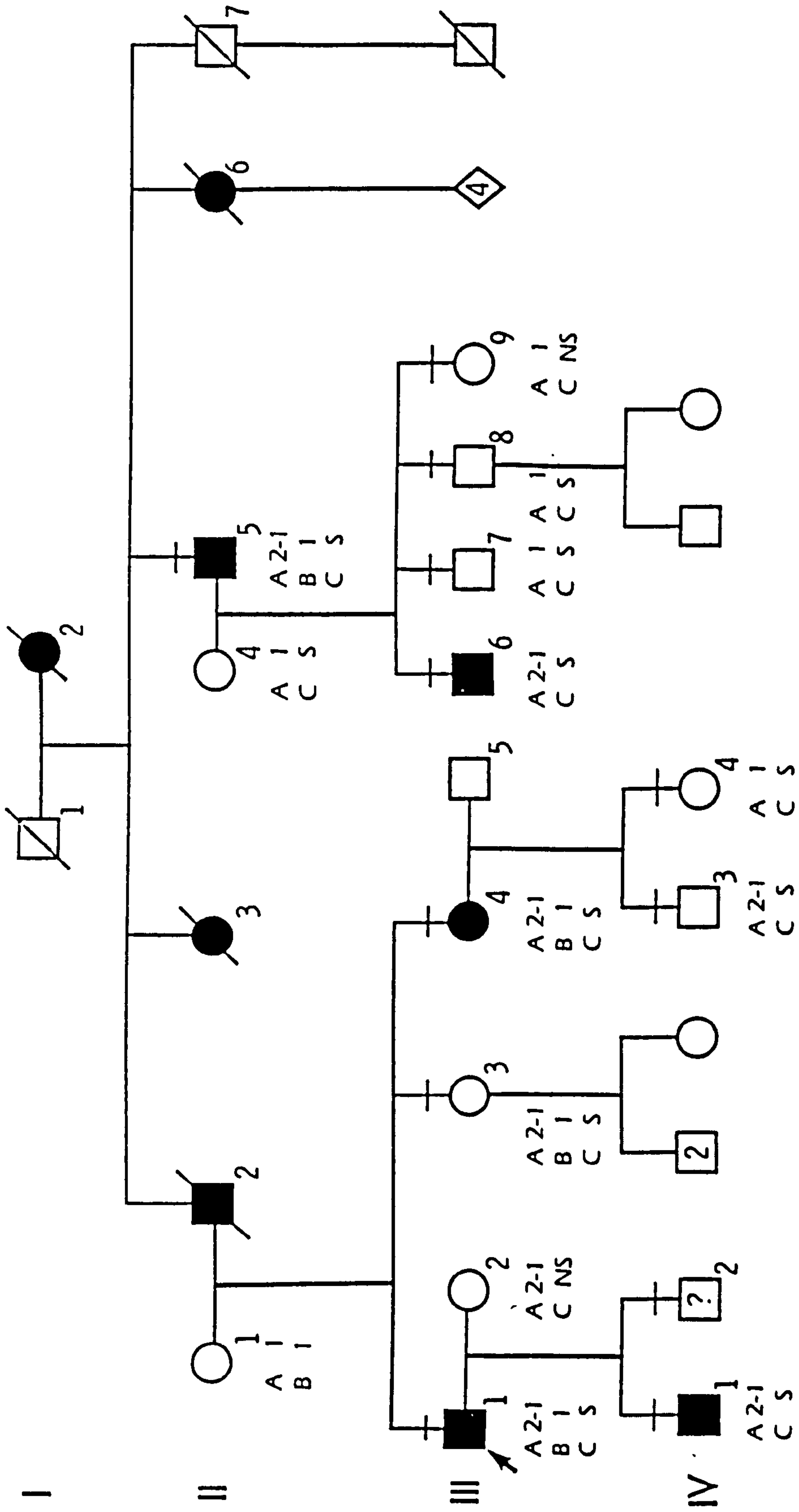
* Multiple epiphyseal
dysplasia

NF70

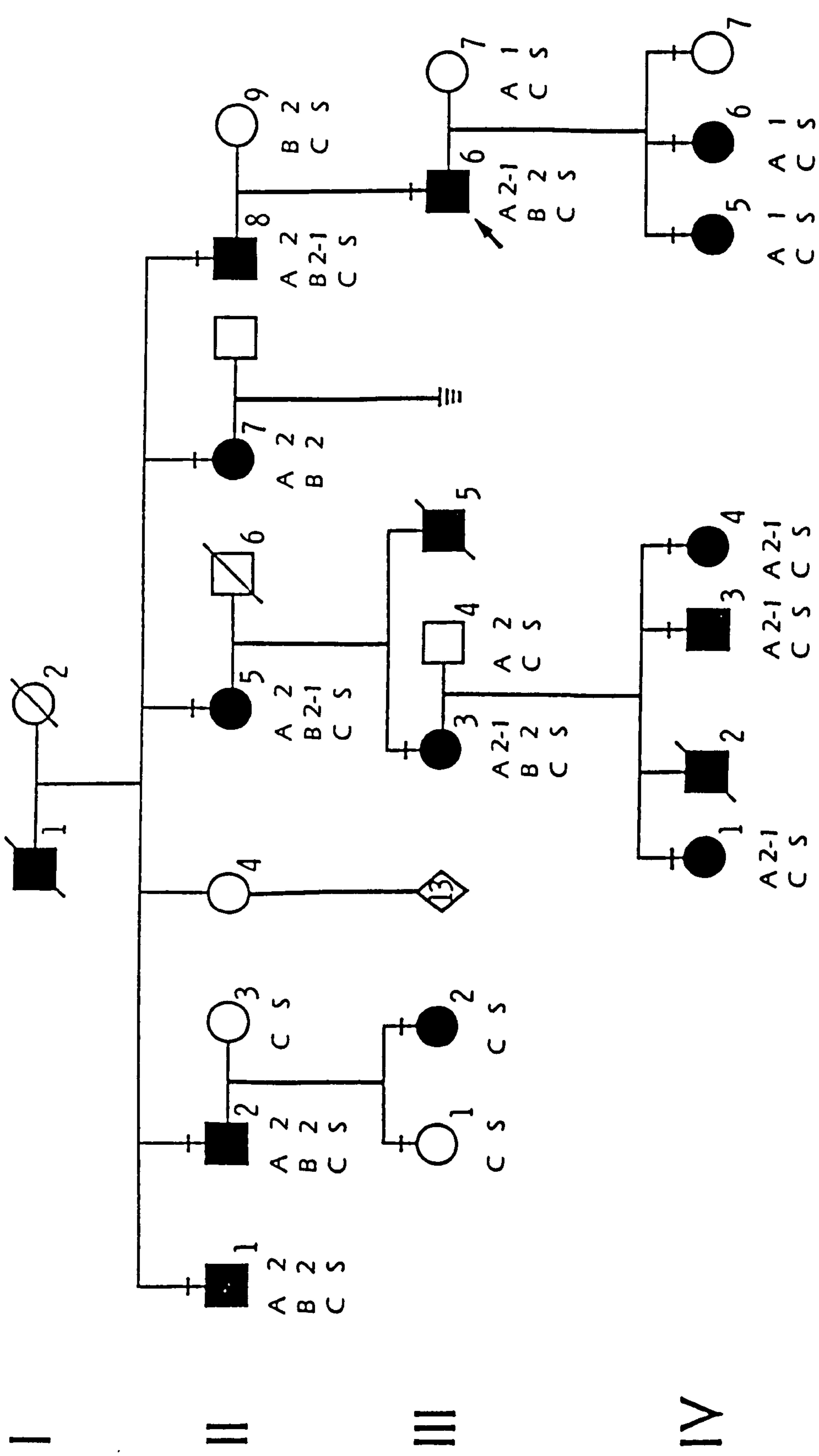


NF71





NF73



APPENDIX C

Study questionnaire used in patient assessment and the computer data entry form for those with NF-1.

NEUROFIBROMATOSIS STUDY

DEPT. OF GENETICS, U.H.W.

Date of assessment:

General information

1. Pedigree No. 1

Person No. 2

2. Sex: Male/female ☐ 3

3. Disease status: Normal/Query/Affected ☐ 4

4. Name: Phone No.

Address:

5. Date of birth:

Day Month Year

5 6 7

6. Occupation:

If appropriate:

Spouses occupation:

Mother's occupation:

Father's occupation

7. Social class:

1. Professional

4. Semi-skilled

2. Intermediate

5. Unskilled ☐ 8

3. Skilled (manual +
non-manual)

6. Other

8. Civil status:

- | | |
|-------------|--------------|
| 1. Single | 4. Separated |
| 2. Married | 5. Widowed |
| 3. Divorced | 6. Engaged |
| | 7. Other |

☐ 9

9. Method of ascertainment:

- | | |
|----------------------|------------------------|
| 1. Hospital records | 7. Neurosurgeon |
| 2. G.P. | 8. Orthopaedic surgeon |
| 3. Neurologist | 9. Plastic surgeon |
| 4. Paediatrician | 10. Dermatologist |
| 5. Geneticist | 11. N.F. Society |
| 6. General physician | 12. Other |

☐ ☐
10

10. G.P. - Name:

Address:

11. Consultant(s) - Name(s):

Hospital:

Hospital No.

SYMPTOM ANALYSIS

Neurofibromata

1. Have you noticed any small lumps on your skin anywhere?
(If no, go to No. 22)

☐ 11

2. How old were you when you first noticed them?

☐ ☐ 12

3. Have you noticed any new ones since then?
(If no, go to No. 17)

☐ 13

4. Have more developed at some times than others, for example:

When you were a teenager? (Yes/No/DK)

When you were pregnant? (Yes/No/DK)

Do they appear at a fairly steady rate? (Yes/No/DK)

☐ 14

☐ 15

☐ 16

5. Have any ever disappeared? (Yes/No/DK)

☐ 17

6. Have any grown in size very much? (Yes/No/DK)
(If YES, delineate (i.e. effect of puberty, pregnancy, sites at which enlarged):

☐ 18

7. Do they bother you in any way?	No	1		
	Pruritis	2	<input type="checkbox"/>	19
	Other	3		

8. Do you have any symptoms before a new one appears?	No	1		
	Pruritis	2	<input type="checkbox"/>	20
	Other	3		

9. Have you ever had one removed?	Yes/No
-----------------------------------	--------

Analysis, re. removal of NFS:				
None removed	1	Cosmetic	4	
Diagnosis	2	Secondary to neurological symptoms	5	<input type="checkbox"/> 21
Malignant change	3	Other	6	

Cafe au lait spots:

1. Have you any birth marks or brown patches on your skin anywhere?	Yes/No	<input type="checkbox"/>	22
(If <u>No</u> go to No. 27)			

2. When did you first notice them?	<input type="checkbox"/> <input type="checkbox"/>	23
------------------------------------	---	----

3. Have any new ones developed since then?	<input type="checkbox"/>	24
--	--------------------------	----

4. When did you last notice a new one?	Age =	<input type="checkbox"/> <input type="checkbox"/>	25
--	-------	---	----

5. Have any ever disappeared?	Yes/No/DK	<input type="checkbox"/>	26
-------------------------------	-----------	--------------------------	----

NEUROLOGICAL SYMPTOMS

1. <u>Headache-</u>	
Do you suffer from headaches?	Yes/No
If <u>Yes</u> , delineate:	
a) site of headache?	
b) kind of pain?	
c) how long does it last?	
d) how often do they occur?	
e) associated symptoms?	

Analysis re. headaches:			
1 No headache	3 Tension		
2 Migraine	4 Other	<input type="checkbox"/>	27

2. Vision - Have you any problems with your eyesight? Yes/No

3. Hearing - Have you any problems with your hearing? Yes/No

Do you have any trouble with dizziness or vertigo? Yes/No

Do you have any buzzing in your ears? Yes/No

4. Epilepsy - Have you ever had a fit? Yes/No

5. Do you have any difficulty in walking? Yes/No

6. Do you have any weakness in your arms? Yes/No

7. Do you have any areas of abnormal sensation - like pins and needles, or areas of numbness? Yes/No

Locomotor symptoms

1. Do you have any problems with your joints? Yes/No

2. Do you have any problems with curvature of the spine? Yes/No

Gastrointestinal symptoms

1. Do you have any abdominal (tummy) pain)? Yes/No

2. Are your bowels regular? Yes/No

Cardiovascular symptoms

1. Have you ever had any problems with your heart? Yes/No

2. Have you ever had high blood pressure? Yes/No/BP never recorded

3. Do you ever get any pain in your chest?

4. Do you ever have episodes where your heart beats rapidly?

5. Do you ever have episodes where you sweat profusely for no apparent reason?

Psychiatric symptoms

Have you ever had problems with your nerves? Yes/No

Birth weight/gestational age

Smoking - Do you smoke? Yes/No
(If yes, how many) =

Other problems

1. Have you any other symptoms that we haven't mentioned? Yes/No

2. Have you had any other problems with your health
that we haven't mentioned? Yes/No

(If male, go to No. 30)

Women only

How old were you when your periods started? Age =

No. of pregnancies	<input type="text"/>	<input type="text"/>	28
No. of livebirths	<input type="text"/>	<input type="text"/>	29
No. of affected children	<input type="text"/>		30
No. of miscarriages	<input type="text"/>		31
No. of stillbirths	<input type="text"/>		32

SCHOOLING AND DEVELOPMENT

Children < 5 years

1. How old when they smiled?
2. How old when they sat unaided?
3. How old when they walked unaided?
4. How old when they first talked?

Children at school

1. Which school do they attend?
2. Are they having any particular problems at school?

Adults

1. Which secondary school did you attend?
2. How did you get on at school? (Tick)
No qualifications O levels
CSE's A levels
3. Did you have any particular problems at school?
4. Did you go on to further education?
1. No

4. Polytechnic

2. Apprenticeship

5. University

3. College F.E.

6. Other

Final analysis (schooling)

- Under 5, normal milestones

1
- Under 5, abnormal milestones

2
- No problems at school

3
- Specific problems at school

4

33

Analysis of Family Data

- Age of father at conception:

34
- Age of mother at conception:

35
- New mutation

1

Mother affected

3
- Father affected

2

Uncertain

4

36

Disease attitudes (only if affected)

1. How old were you when you first realised you
(or your child) had NF? Age =
2. What happened at that time to make you realise?
(N.B. Comment if never seen by a doctor)
3. Do you understand how NF is passed on in families?
4. Have you ever asked a doctor for advice about how
NF is passed on in families?
5. What have been the main problems that having NF
has caused you?

EXAMINATION

1. Height = cms
2. Centile for 1.
3. Head circumference = cms
4. Centile for 3.
5. Weight = Kg
6. Skin pigmentation
 - a) No. of CAL spots > 1.5 cms:
 - b) If < 18 years, No. of CAL spots > 0.5cms <1.5 cms
 - c) Axillary freckling: None/Bilateral/R/L

Average size of freckles =

 - d) Other areas of freckling = Yes/No

Sites:

 - e) CAL spots overlapping plexiform NFS - see map.
7. Neurofibromas:
 - a) Nodular -
 1. None 4. > 100
 2. Few < 10
 3. Scanty 10-99

Form of NF: cutaneous/subcutaneous/Peduncular
 - b) Plexiform

No = YES/NO
 - c) Areolar NFs YES/NO

No R. breast

No L breast

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<div style="border: 1px solid black; display: inline-block; width: 30px; height: 30px; vertical-align: middle;"></div>	38
<div style="border: 1px solid black; display: inline-block; width: 30px; height: 30px; vertical-align: middle;"></div>	39
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<div style="border: 1px solid black; display: inline-block; width: 30px; height: 30px; vertical-align: middle;"></div>	44
<div style="border: 1px solid black; display: inline-block; width: 30px; height: 30px; vertical-align: middle;"></div>	45
<div style="border: 1px solid black; display: inline-block; width: 30px; height: 30px; vertical-align: middle;"></div>	46
<div style="border: 1px solid black; display: inline-block; width: 30px; height: 30px; vertical-align: middle;"></div>	47
<div style="border: 1px solid black; display: inline-block; width: 30px; height: 30px; vertical-align: middle;"></div>	48

Women only

How old were you when they appeared?

What happened to them during pregnancy?

Neurofibromas

Facial appearance, 'disfiguring' = Yes/No

Neurofibromas/area: + < 10

++ 10-50 +++ >50

Face =

Trunk =

R. arm =

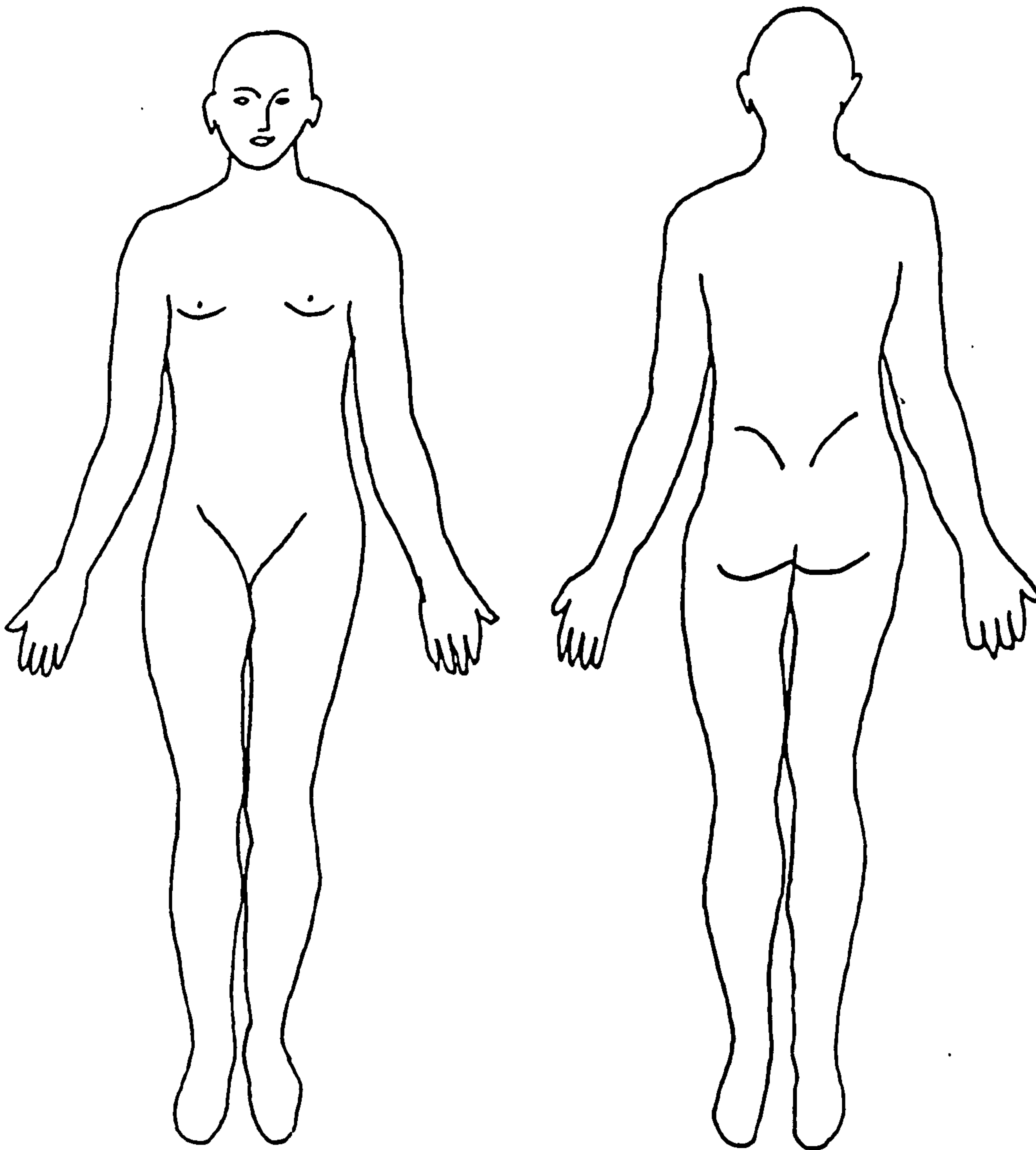
L. arm =

R. Leg =

L. Leg =

Soles =

Palms =

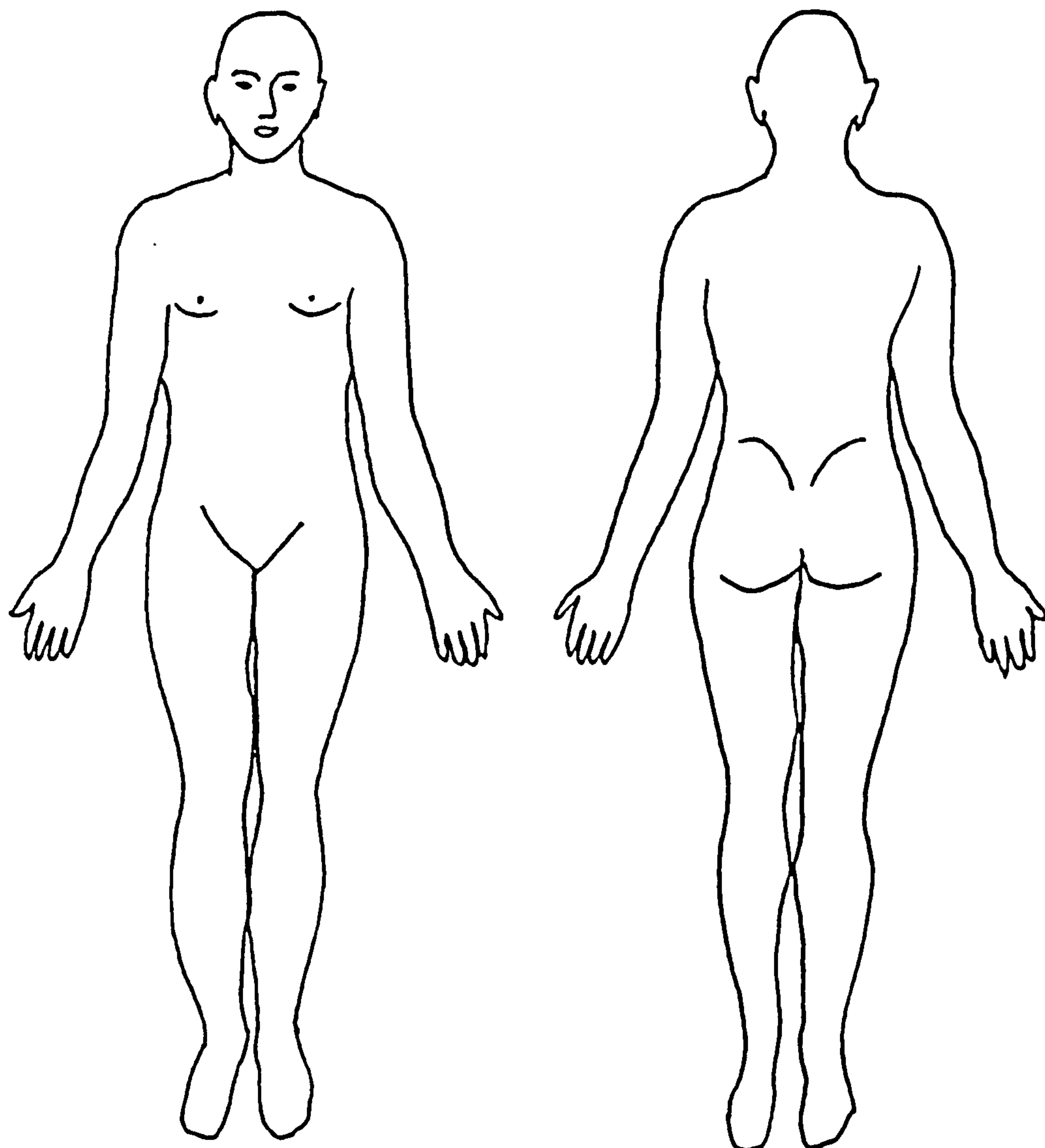


Patient's appearance

Cafe au laits

Draw in on figures if < 18 years.

Draw in pigmentation in relation to plexiform neurofibromata



8. Skeletal examination

- | | |
|--|------------------|
| 1. Skull - N/ABN | 3. Limbs - N/ABN |
| 2. Back - N/ABN. If scoliosis present, note cervical/thoracic/lumbar | |

9. CVS examination

P =	B.P. =	E.	S.		
Apex =	HS =				
		RAD.	FEM.	ABDO	BRUITS
If B.P. raised (R					
(L					

10. Neurological Examination

- a) Speech:
- b) Mental state:
- c) Cranial nerves

II-	R	L	III, IV, VI:
V.A.			
V.F.			
Pupils			V:
Fundi			VII:
VIII:			IX, X, XI:
			XII:

d) Motor

Tone = N/ABN	Coord. = N/ABN
Power = N/ABN	
Reflexes = N/ABN	

e) Sensation - N/ABN

11. Lisch nodules

	Naked eye app	Slit lamp No.	Slit lamp colour
--	------------------	------------------	---------------------

R eye

L eye

Lisch n. R. eye ☐☐ 49

Lisch n. L. eye ☐☐ 50

Final analyses, complications

1. Neurological:

None	1	NF with neurol complication	7
Epilepsy	2	Definite low IQ	8
Optic glioma	3	Learning difficulties etc.	9
Accoustic neuroma	4	Other	10
Meningioma	5		
Other tumours	6		

☐☐☐☐ 51

2. Skeletal abnormalities

Normal	1
Skull defects	2
Pseudoarthroses	3
Scoliosis/kyphosis	4

☐ 52

3. Other NF complications

None	1	Hypertension unrelated to 4	5
Visceral tumours	2	Cardiovascular problems	6
Constipation	3	Other	7
Endocrine complications	4		

☐☐ 53

NEUROFIBROMATOSIS SURVEY

PATIENT SUMMARY

1. Study area:	Yes/No
2. Pedigree number	_____
Person number	_____
3. Name:	
4. Sex:	M / F
5. Status:	UN/RN/Q/A
6. Date of birth:	—/—/—
7. Social class:	—
8. Civil status:	—
9. Education:	—
10. Height	_____
11. Centile for (10)	L/N/S
12. Head circumference	_____
13. Centile for (12)	L/N/S
14. Weight	_____
15. Skin pigmentation:	
a) No of CAL spots > 1.5cm	_____
b) <18 years, no. of CAL spots >0.5 cm	_____
c) Freckling:	
Axillary	Y/N
Submammary	Y/N
Groins	Y/N
Other	Y/N
d) Age CALs first noted	_____
e) Have any disappeared?	Y/N/DK

16. a) Dermal neurofibromas	—
b) Age at first appearance	—
c) Symptoms	-
d) Any symptoms before a neurofibroma appears?	—
e) Removal of NFs	—
f) Facial appearance	O/N
g) Did more appear when pregnant?	Y/N
h) Areolar neurofibromas	Y/N
i) Plexiform neurofibromas	Y/N
j) Site of (i): 1. Face	—
2. Trunk	—
3. Limb	—
k) Age at appearance of (i)	—
l) Skin hypertrophy overlying (i)	Y/N
m) Bone hypertrophy associated with (i)	Y/N
n) Hair overlying (i)	Y/N
o) Operation for removal of (i)	Y/N
17. Lisch nodules: No. right eye	—
No. left eye	—
18. Neurological complications	—
19. Skeletal abnormalities	—
20. Other NF complications	—
21. Genetics: a) Status	—
b) Father	—
Mother	—
c) Age at diagnosis	—
d) Diagnosed 2° to study	Y/N
e) Genetic counselling	Y/N

22. Grade of NF	—
23. Method of ascertainment	—
24. GP analysis	—
25. Regularly followed-up for NF	Y/N